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(57) Abstract

The present invention provides bacterial immunogenic agents for adminstration to humans and non–human animals to stimulate an immune response. It particularly relates to the vaccination of mammalian species with pneumococcal derived polypeptides that include an alpha helix but exclude a choline binding region as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. In another aspect the invention provides antibodies against such proteins and protein complexes that may be used as diagnostics and/or as protective/treatment agents for pathogenic bacterial species.

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DERIVATIVES OF PNEUMOCOCCAL CHOLINE BINDING PROTEINS FOR VACCINES

This application claims the benefit of U.S. Prov. Appl'n Serial No. 60/085,743, filed May 15, 1998 and U.S. Prov. Appl'n Serial NO 60/080,878, filed April 7, 1998.

This invention relates generally to the field of bacterial antigens and their use, for example, as immunogenic agents in humans and animals to stimulate an immune response. More specifically, it relates to the vaccination of mammalian species with a polypeptide comprising an alpha helix-forming polypeptide obtained from a choline binding polypeptide as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. Further, the invention relates to antibodies and antagonists against such polypeptides useful in diagnosis and passive immune therapy with respect to diagnosing and treating such pneumococcal infections.

In a particular aspect, the present invention relates to the prevention and treatment of pneumonococcal infections such as infections of the middle ear, nasopharynx, lung and bronchial areas, blood, CSF, and the like, that are caused by pneumonococcal bacteria. In this regard, certain types of *Streptococcus pneumoniae* are of particular interest.

S. pneumoniae is a gram positive bacteria which is a major causative agent in invasive infections in animals and humans, such as sepsis, meningitis, otitis media and lobar pneumonia (Tuomanen, *et al.* NEJM 322:1280-1284 (1995)). As part of the infective process, pneumococci

readily bind to non-inflamed human epithelial cells of the upper and lower respiratory tract by binding to eukaryotic carbohydrates in a lectin-like manner (Cundell *et al.*, Micro. Path. 17:361-374 (1994)). Conversion to invasive pneumococcal infections for bound bacteria may involve the local generation of inflammatory factors which may activate the epithelial cells to change the number and type of receptors on their surface (Cundell, *et al.*, Nature, 377:435-438 (1995)). Apparently, one such receptor, platelet activating factor (PAF) is engaged by the pneumococcal bacteria and within a very short period of time (minutes) from the appearance of PAF, pneumococci exhibit strongly enhanced adherence and invasion of tissue. Certain soluble receptor analogs have been shown to prevent the progression of pneumococcal infections (Idanpaan-Heikkila *et al.*, J. Inf. Dis., 176:704-712 (1997)).

A family of choline binding proteins (CBPs), which are non-covalently bound to phosphorylcholine, are present on the surface of pneumococci and have a non-covalent association with teichoic acid or lipoteichoic acid. An example of such family is choline binding protein A (CbpA), an approximately 75kD weight type of CBP which includes a unique N-terminal domain, a proline rich region, and a C-terminal domain comprised of multiple 20 amino acid repeats responsible for binding to choline. A segment of the N-terminal portion of CbpA protein forms an alpha helix as part of its three-dimensional structure.

Accordingly, it is an object of the present invention to provide a polypeptide having broad protection against pneumococcal infections.

Definitions

In order to facilitate understanding of the description below and the examples which follow certain

frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically

synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

"HPS portion" as used herein refers to an amino acid sequence as set forth in SEQ ID NO:2 for a choline binding protein ("CBP") of a pneumococcal bacteria that may be located amino terminal with respect to the proline rich portion of the overall amino acid sequence for such CBP.

The terms "identity", "% identity" or "percent identity" as utilized in this application refer to a calculation of differences between two contiguous sequences which have been aligned for "best fit" (to provide the largest number of aligned identical corresponding sequence elements, wherein elements are either nucleotides or amino acids) and all individual differences are considered as individual difference with respect to the identity. In this respect, all individual element gaps (caused by insertions and deletions with respect to an initial sequence ("reference sequence")) over the length of the reference sequence and individual substitutions of different elements (for individual elements of the reference sequence) are considered as individual differences in calculating the total number of differences between two sequences. Individual differences may be compared between two sequences where an initial

sequences (reference sequence) has been varied to obtain a variant sequence (comparative sequence) or where a new sequence (comparative sequence) is simply aligned and compared to such a reference sequence. When two aligned sequences are compared all of the individual gaps in BOTH sequences that are caused by the "best fit" alignment over the length of the reference sequence are considered individual differences for the purposes of identity. If an alignment exists which satisfies the stated minimum identity, then a sequence has the stated minimum identity to the reference sequence. For example, the following is a hypothetical comparison of two sequences having 100 elements each that are aligned for best fit wherein one sequence is regarded as the "reference sequence" and the other as the comparative sequence. All of the individual alignment gaps in both sequences are counted over the length of the reference sequence and added to the number of individual element substitution changes (aligned elements that are different) of the comparative sequence for the total number of element differences. The total number of differences (for example 7 gaps and 3 substitutions) is divided by the total number of elements in the length of the reference sequence (100 elements) for the "percentage difference" (10/100). The resulting percentage difference (10%) is subtracted from 100% identity to provide a "% identity" of 90% identity. For the identity calculation all individual differences in both sequences are considered in the above manner over a discrete comparison length (the length of the reference sequence) of two best fit aligned sequences to determine identity. Thus, no algorithm is necessary for such an identity calculation.

"Isolated" in the context of the present invention with respect to polypeptides and/or polynucleotides means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living organism

is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

Summary of the Invention

In one aspect the present invention relates to a vaccine for treating or preventing pneumococcal bacterial infections which utilizes as an immunogen at least one polypeptide truncate of a pneumococcal surface-binding protein, analog, or variant having a highly conserved immunogenic alpha-helical portion (corresponding generally to a "consensus" amino acid sequence as set forth in SEQ ID NO:1) with respect to different types of pneumococcal bacteria, which polypeptide does not include a choline-binding portion. Preferably, the C-terminal choline-binding portion is absent from such polypeptides. More preferred are such polypeptides wherein the HPS amino acid sequence is also absent. Even further preferred are polypeptides wherein the highly conserved immunogenic alpha-helical portion corresponding generally to a "consensus" amino acid sequence as set forth in SEQ ID NO:1 also corresponds generally to the amino acid sequence as set forth in SEQ ID NO:19 (amino acids 1 to 103 of SEQ ID NO:19 are identical to amino acids 1 to 103 of SEQ ID NO:1). Also preferred as vaccines are recombinantly-produced, isolated polypeptides that are missing both an HPS portion and the choline-binding portion.

More preferred as vaccines are one or more polypeptide truncates of pneumococcal surface-binding proteins, analogs or variants including a single highly conserved alpha-helix immunogenic portion with respect to

different types of pneumococci, which polypeptides do not include a C-terminal choline-binding portion. Further preferred are isolated recombinantly produced polypeptides having such structure. Also preferred are such polypeptides that do not include either a C-terminal choline-binding portion or a HPS portion.

The present invention further provides a vaccine comprising a polypeptide including an immunogenic portion that is capable of forming an alpha helix, which polypeptide includes a sequence that has at least 85% identity and preferably at least 87% identity to the amino acid sequence of SEQ ID NO:1, wherein the isolated polypeptide does not include a C-terminal choline-binding portion. Further preferred are such polypeptides that comprise a polypeptide sequence that has at least 85% identity and preferably at least 87% identity to an amino acid sequence according SEQ ID NO:19. Preferably, the sequence of the isolated polypeptide includes neither an HPS portion (SEQ ID NO:2) nor a C-terminal choline-binding portion. Further preferred are isolated recombinantly produced polypeptides having such structure. In particular, such polypeptides corresponding to alpha helical structures of different types of *S. pneumoniae* bacteria are contemplated. Particularly preferred are the serotypes 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F of such *S. pneumoniae* bacteria. Examples of such serotypes of bacteria are readily available from standard ATCC catalogs.

In an additional aspect, the present invention further provides a vaccine against *S. pneumoniae* comprising a synthetic or recombinant polypeptide comprising a plurality of alpha-helical portions, each derived from different naturally occurring *S. pneumoniae* choline-binding polypeptides wherein such alpha-helical portions have at least 85% identity to the amino acid sequence of SEQ ID NO:1, and wherein the isolated

polypeptide does not include a choline-binding portion. Further preferred are those wherein the amino acid sequence for the alpha-helix areas is at least 85% identical to the amino acid sequence of SEQ ID NO:19. Preferably, such synthetic polypeptide includes neither a HPS portion nor a choline-binding portion. Analogs and variants of such chain structure polypeptides wherein such alpha helical portions may be synthetic variant amino acid sequences (or may be a mixture of naturally occurring and variant sequences) are also contemplated and embraced by the present invention. In a preferred aspect, chain vaccines polypeptides having at least ten different alpha helical structures corresponding to *S. pneumoniae* serotypes 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F are provided. Further preferred are polypeptides including at least fifteen of such alpha-helical structures, more preferred are polypeptides including at least 20 such alpha-helical structures and more preferred are polypeptides including at least one alpha-helical structure corresponding to each of the *S. pneumoniae* serotypes 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F. Another preferred polypeptide comprises each of the alpha helical structures from the amino acid sequences of SEQ ID NOS:3-18 which correspond to SEQ ID NO:1.

In another aspect, the invention relates to passive immunity vaccines formulated from antibodies against a polypeptide including a highly conserved immunogenic portion with respect to different types of pneumococcal bacteria which portion is capable of forming an alpha-helix having the hereinbefore described identity to the amino acid sequence of SEQ ID NO:1, which polypeptide does not include a C-terminal choline-binding portion, wherein said antibodies will bind to at least one *S. pneumoniae* species. Preferably, if such polypeptide is a truncate of a native pneumococcal surface-binding protein both its HPS portion (where applicable) and its choline-binding portion

are absent from such polypeptide. Such passive immunity vaccines can be utilized to prevent and/or treat pneumococcal infections in immunocompromised patients, patients having an immature immune system (such as young children) or patients who already have an ongoing infection. In this manner, according to a further aspect of the invention, a vaccine can be produced from a synthetic or recombinant polypeptide wherein the polypeptide includes the conserved alpha helical portions of two or more different choline binding polypeptides of *S. pneumoniae*.

This invention also relates generally to the use of an isolated polypeptide having a highly conserved immunogenic portion with respect to different types of pneumococcal bacteria which portion is capable of forming an alpha-helix (corresponding generally to SEQ ID NO:1 or to SEQ ID NO:19) wherein the isolated polypeptide does not include a choline-binding portion, to raise antibodies in non-human mammalian species useful, for example, as diagnostic reagents and vaccines.

In yet another aspect, the present invention relates to the production of a polypeptide including a highly conserved immunogenic portion with respect to different types of pneumococcal bacteria which portion is capable of forming an alpha-helix whose sequence corresponds generally to the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:19, wherein the isolated polypeptide does not include a choline-binding portion. Preferably, such recombinant production is of a truncated native pneumococcal surface-binding polypeptide wherein both the HPS portion (where applicable) and the choline-binding portion are absent.

In still another aspect, the present invention provides an isolated choline-binding polypeptide, wherein the non-choline binding region of such polypeptide has at least 90% identity to the corresponding amino acid

sequence portion of a naturally occurring pneumococcal surface-binding protein which is a member selected from the group consisting of SEQ ID NOS:3-18. The invention relates to fragments of such polypeptides which include at least the conserved alpha-helical portion corresponding generally to SEQ ID NO:1, and which has at least 85% identity thereto, wherein the isolated polypeptide preferably is free of a choline binding region.

In another aspect the present invention provides an isolated polypeptide comprising an amino acid sequence which has at least 90% identity to one of the amino acid sequences selected from the group consisting of SEQ ID NO:3-18. Preferably, such isolated polypeptide comprises an amino acid sequence which has at least 95% identity, and more preferably 97% identity, to one of the amino acid sequences selected from the group consisting of SEQ ID NO:3-18. The invention further relates to fragments of such polypeptides.

In a yet further aspect, the present invention provides a *S. pneumoniae* CBP polypeptide encoded by a polynucleotide that will hybridize under highly stringent conditions to the complement of a polynucleotide encoding a polypeptide having an amino acid selected from the group consisting of SEQ ID NOS:1 and 3-18. Particularly preferred are polypeptides comprising an amino acid sequence segment that is at least 90% identical to the amino acid sequence of SEQ ID NO:1. Further preferred are such polypeptides comprising a contiguous amino acid sequence that has at least 95% identity with respect to the amino acid sequence of SEQ ID NO:1. And, even more preferred are polypeptides comprising an amino acid sequence that has at least 97% identity with respect to the amino acid sequence of SEQ ID NO:1.

In another aspect the present invention provides polynucleotides which encode the hereinabove described polypeptides of the invention. The polynucleotide of the

present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The polynucleotides which encode polypeptides including the amino acid sequences of at least one of SEQ ID NOS:3-18 (or polypeptides that have at least 90% identity to the amino acid sequences of such polypeptides) may be one of the coding sequences shown in SEQ ID NOS:20-35 or may be of a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptides as the DNA of SEQ ID NOS:20-35.

The polynucleotides which encode the polypeptides of SEQ ID NOS:3-18 may include: only the coding sequence for the polypeptide; the coding sequence for the polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide. The polypeptides encoded may comprise just a single alpha-helical portion or multiple alpha-helical portion and may independently or collectively include N-terminal sequences 5' of such alpha helical areas and/or sequences corresponding to the "X" structures or proline rich areas (as set forth in Figure 1, for example).

The invention further relates to a polynucleotide comprising a polynucleotide seqeunce that has at least 95% identity and preferably at least 97% identity to a polynucleotide encoding one of the polypeptides comprising SEQ ID NO:3-18. The invention further relates to fragments of such polynucleotides which include at least the portion of the polynucleotide encoding the polypeptide sequence corresponding to SEQ ID NO:1.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes

only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence. In particular, the polypeptides may include any or all of the types of structures set forth schematically in Figure 1.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides including the amino acid sequences of SEQ ID NOS:3-18. The variants of the polynucleotides may be a naturally occurring allelic variant of the polynucleotides or a non-naturally occurring variant of the polynucleotides. Complements to such coding polynucleotides may be utilized to isolate polynucleotides encoding the same or similar polypeptides. In particular, such procedures are useful to obtain alpha helical coding segments from different serotypes of *S. pneumoniae*, which is especially useful in the production of "chain" polypeptide vaccines containing multiple alpha helical segments.

Thus, the present invention includes polynucleotides encoding polypeptides including the same polypeptides as shown in the Sequence Listing as SEQ ID NOS:3-18 as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of SEQ ID NOS:3-18. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in the Sequence Listing as SEQ ID NOS:20-35. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be, for example, a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides (hybridization target sequences) which hybridize to the complements of the hereinabove-described sequences if there is at least 70% and preferably 80% identity between the target sequence and the complement of the sequence to which the target sequence hybridizes, preferably at least 85% identity. More preferred are such sequences having at least 90% identity, preferably at least 95% and more preferably at least 97% identity between the target sequence and the sequence of complement of the polynucleotide to which it hybridizes. The invention further relates to the complements to both the target sequence and to the polynucleotide sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NOS:3 to 18. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the complements of the hereinabove-described polynucleotides as well as to those complements. As herein used, the term "stringent conditions" means hybridization will occur with the complement of a polynucleotide and a corresponding sequence only if there is at least 95% and preferably at least 97% identity between the target sequence and the sequence of complement of the polynucleotide to which it hybridizes. The polynucleotides which hybridize to the complements of the hereinabove described polynucleotides

in a preferred embodiment encode polypeptides which retain an immunogenic portion that will cross-react with an antibody to at least one of the polypeptides having a sequence according to SEQ ID NOS:3-18, or to a polypeptide that includes an amino acid sequence which has at least 85% identity to that of SEQ ID NO:1.

In a still further aspect, the present invention provides for the production of such polypeptides and vaccines as set forth above having a histidine label (or other suitable label) such that the full-length proteins, truncates, analogs or variant discussed above can be isolated due to their label.

In another aspect the present invention relates to a method of prophylaxis and/or treatment of diseases that are mediated by pneumococcal bacteria that have surface-binding CBP proteins. In particular, the invention relates to a method for the prophylaxis and/or treatment of infectious diseases that are mediated by *S. pneumoniae* that have a CBP surface-binding protein that forms an alpha helix (comprising a sequence that has at least an 85% identity to the amino acid sequence of SEQ ID NO:1). In a still further preferred aspect, the invention relates to a method for the prophylaxis and/or treatment of such infections in humans.

In still another aspect the present invention relates to a method of using one or more antibodies (monoclonal, polyclonal or sera) to the polypeptides of the invention as described above for the prophylaxis and/or treatment of diseases that are mediated by pneumococcal bacteria that have CBP surface-binding proteins. In particular, the invention relates to a method for the prophylaxis and/or treatment of infectious diseases that are mediated by *S. pneumoniae* CBP proteins which include an alpha helical portion having the hereinbefore described identity to the consensus sequence of SEQ ID NO:1. In a still further preferred aspect, the

invention relates to a method for the prophylaxis and/or treatment of otitis media, nasopharyngeal, bronchial infections, and the like in humans by utilizing antibodies to the alpha-helix containing immunogenic polypeptides of the invention as described above.

Brief Description of Drawings

Figure 1 is a diagram of a pneumococcal CBP protein which shows from the N-terminal to the C-terminal, respectively, (a) a N-terminal sequence, (b) one of a potential alpha-helical forming area conserved segment (R1) that may not be present in some CBP polypeptides, (c) an optional small bridging sequence of amino acids that may bridge two conserved alpha-helical segments (X), (d) a second of a potential alpha-helical forming area consensus sequence (R2) related to the first consensus sequence (which corresponds to SEQ ID NO:1), (e) a proline rich area sequence, (f) a choline binding repeats area, and (e) a C-terminal tail sequence. Where relevant, an optional HPS sequence may naturally occur 5' of the proline rich sequence and 3' of the R1, X, and/or R2 areas.

Figure 2 reports the results for passive immunity protection against 1600 cfu virulent serotype 6B *S. pneumoniae* SP317 (in mice) that was provided by day 31 rabbit antisera to a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). Eighty percent of the mice immunized with the truncate antisera prior to challenge survived the 14 day observation period. By contrast, all mice immunized with a control sera (pre-immune rabbit sera) were dead by day 7.

Figure 3 reports the results for passive immunity protection against 3450 cfu virulent serotype 6B *S. pneumoniae* SP317 (in mice) that was provided by day 52 rabbit antisera to a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). One hundred percent of the mice immunized with the truncate antisera prior to challenge survived the 10 day observation period. By

contrast, ninety percent of the mice immunized with a control sera (pre-immune rabbit sera) were dead at day 10.

Figure 4 reports the results for passive immunity protection against 580 cfu virulent serotype 6B *S. pneumoniae* SPSJ2 (in mice) that was provided by day 31 rabbit antisera to a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). Fifty percent of the mice immunized with the truncate antisera prior to challenge survived the 10 day observation period. By contrast, all mice immunized with a control sera (pre-immune rabbit sera) were dead by day 8.

Figure 5 reports the results for active immunity protection against 560 cfu virulent serotype 6B *S. pneumoniae* SPSJ2 (in mice) that was provided by immunization with a pneumococcal CBP truncate polypeptide, NR1X (truncate missing the second conserved alpha-helical area R2, as well as both the proline and the choline binding areas). Eighty percent of the mice actively immunized with the NR1X CBP truncate prior to challenge survived the 14 day observation period. By contrast, all mice immunized with a control (sham mice) of PBS and adjuvant were dead by day 8.

Figure 6 reports the results for active immunity protection against 680 cfu virulent serotype 6B *S. pneumoniae* SPSJ2 (in mice) that was provided by immunization with a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). Fifty percent of the mice actively immunized with the NR1XR2 CBP truncate prior to challenge survived the 14 day observation period. By contrast, all mice immunized with a control (SP90) protein and adjuvant were dead by day 9.

Figure 7 is an alignment report of the amino terminus of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:36). One letter codes are utilized to represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 8 shows the sequence pair distances for the amino acid sequences as described for Figure 7 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 7.

Figure 9 is an alignment report for a first helical region in the amino acid sequences of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:38). One letter codes are utilized to represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 10 shows the sequence pair distances for the amino acid sequences as described for Figure 9 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 9.

Figure 11 is an alignment report for the region X in the amino acid sequences of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the

comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:37). One letter codes are utilized to represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 12 shows the sequence pair distances for the amino acid sequences as described for Figure 11 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 11.

Figure 13 is an alignment report for the second helical region A in the amino acid sequences of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:1). One letter codes are utilized to represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 14 shows the sequence pair distances for the amino acid sequences as described for Figure 13 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 13.

Figure 15 is an alignment report for the second helical region B in the amino acid sequences of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:19). One letter codes are utilized to

represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 16 shows the sequence pair distances for the amino acid sequences as described for Figure 15 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 15.

Detailed Description of the Invention

In accordance with an aspect of the present invention there is provided a vaccine to produce a protective response against *S. pneumoniae* infections which employs a polypeptide which comprises a member selected from the group consisting of:

(a) an amino acid sequence which produces an alpha helical structure and which is at least 85% identical to the amino acid sequence of SEQ ID NO:1 and which is free of a choline binding region, and

(b) an isolated truncate of a naturally occurring *S. pneumoniae* polypeptide that comprises an alpha helical portion that has at least 85% identity to the amino acid sequence of SEQ ID NO:1 and is free of a choline binding region,

(c) an isolated truncate of a naturally occurring *S. pneumoniae* polypeptide that comprises an alpha helical portion that has at least 90% identity to the amino acid sequence of SEQ ID NO:19 and is free of a choline binding region. In a preferred aspect, such isolated truncate polypeptide is a member selected from the group consisting of SEQ ID NOS:3-18 and said isolated polypeptide is free of a choline binding region and, if relevant, a HPS region; or a fragment thereof which includes at least the alpha helical segment which corresponds to the consensus sequence of SEQ ID NO:1. Particularly preferred are

vaccines which utilize such truncate polypeptides that include at least such alpha helical area or utilize a recombinant immunogen polypeptide comprising at least two of such alpha-helical segments. Such polypeptide may be a recombinant polypeptide containing multiple alpha-helical areas from one or more trucates. Further preferred are recombinant immunogen polypeptides comprising at least two alpha-helical areas corresponding to the alpha helical areas of two or more truncates from different types of pneumococcal bacteria. Such polypeptide may be a recombinant polypeptide containing multiple alpha-helical areas from one or more different types of pneumococcal bacteria.

In accordance with the present invention, there is provided an isolated polypeptide comprising a truncated surface-binding polypeptide derived from *S. pneumoniae*, said isolated polypeptide containing an alpha-helical area whose amino acid sequence corresponds generally to the amino acid sequence of SEQ ID NO:1, but free of a choline binding area. Preferably, said isolated polypeptide also omits any naturally occurring repeats of the alpha-helical forming area and omits any HPS amino acid sequence that may be present.

It is an object of the present invention to utilize as immunogenic composition for a vaccine (or to produce antibodies for use as a diagnostic or as a passive vaccine) comprising an immunogenic polypeptide comprising a pneumococcal surface-binding polypeptide with an alpha helical portion from which a choline binding region has been omitted. In one embodiment, such truncated proteins (naturally or recombiantly produced, as well as functional analogs) from *S. pneumoniae* bacteria are contemplated. Even more particularly, *S. pneumoniae* polypeptides having a single alpha helical portion that omit any HPS areas that occur and choline binding areas of the native protein are contemplated.

A particularly preferred embodiment of such an immunogenic composition is for use as a vaccine (or as an immunogen for producing antibodies useful for diagnostics or vaccines) wherein the active component of the immunogenic composition is an isolated polypeptide comprising at least one member selected from the group consisting of:

(a) an amino acid sequence which is selected from SEQ ID NOS:3-19,

(b) a polypeptide which has at least 90% identity to (a), preferably at least 95% identity to (a), and even more preferred at least 97% identity to (a), or

(c) a fragment of (a) or (b) wherein such fragment includes at least one alpha helical portion that corresponds to the consensus sequence which is SEQ ID NO:1 and said fragment does not comprise a choline binding region. Preferably, such vaccines utilize a polypeptide that contains neither a choline binding region nor an HPS region that occurs as part of the amino acid sequences in the native proteins.

In another preferred embodiment, there is provided a vaccine which includes at least one isolated polypeptide which includes an amino acid sequence which has at least 85% identity (preferably 87% identity and more preferably at least 90% identity) to SEQ ID NO:1, which isolated polypeptide is free of a choline binding portion and, where applicable, is also preferably free of an HPS portion. The preferred polypeptide may also include one or more of the N-terminal sequences that are located 5' of the alpha helical areas in the polypeptides having an amino acid sequence selected from the group consisting of SEQ ID NOS:3-18, or the like. The polypeptide truncate may also include one or more of the proline regions (region "P" in Figure 1) and/or the spanning region (region "X" in Figure 1).

In another aspect of the invention, such an immunogenic composition may be utilized to produce

antibodies to diagnose pneumococcal infections, or to produce vaccines for prophylaxis and/or treatment of such pneumococcal infections as well as booster vaccines to maintain a high titer of antibodies against the immunogen(s) of the immunogenic composition.

While other antigens have been contemplated to produce antibodies for diagnosis and for the prophylaxis and/or treatment of pneumococcal infections, there is a need for improved or more efficient vaccines. Such vaccines should have an improved or enhanced effect in preventing bacterial infections mediated pneumococci having surface-binding polypeptides. Further, to avoid unnecessary expense and provide broad protection against a range of pneumococcal serotypes there is a need for polypeptides that comprise an immunogenic amino acid sequence corresponding to a portion of pneumococcal surface-binding polypeptides that is a highly conserved portion among various types of pneumococci. Preferably, such polypeptides avoid the inclusion of amino acid sequences corresponding to other portions of the native polypeptides, such as the choline binding region and/or the HPS region.

There is a need for improved antigenic compositions comprising highly conserved portions of polypeptides that bind to the surface of pneumococcal bacteria for stimulating high-titer specific antisera to provide protection against infection by pathogenic pneumococcal bacteria and also for use as diagnostic reagents.

In such respect, truncated polypeptides, functional variant analogs, and recombinantly produced truncated polypeptides of the invention are useful as immunogens for preparing vaccine compositions that stimulate the production of antibodies that can confer immunity against pathogenic species of bacteria. Further, preparation of vaccines containing purified proteins as antigenic ingredients are well known in the art.

Generally, vaccines are prepared as injectables, in the form of aqueous solutions or suspensions. Vaccines in an oil base are also well known such as for inhaling. Solid forms which are dissolved or suspended prior to use may also be formulated. Pharmaceutical carriers are generally added that are compatible with the active ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to, water, saline solutions, dextrose, or glycerol. Combinations of carriers may also be used.

Vaccine compositions may further incorporate additional substances to stabilize pH, or to function as adjuvants, wetting agents. or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

Vaccines are generally formulated for parenteral administration and are injected either subcutaneously or intramuscularly. Such vaccines can also be formulated as suppositories or for oral administration, using methods known in the art.

The amount of vaccine sufficient to confer immunity to pathogenic bacteria is determined by methods well known to those skilled in the art. This quantity will be determined based upon the characteristics of the vaccine recipient and the level of immunity required. Typically, the amount of vaccine to be administered will be determined based upon the judgment of a skilled physician. Where vaccines are administered by subcutaneous or intramuscular injection, a range of 50 to 500 μg purified protein may be given.

The term "patient in need thereof" refers to a human that is infected with, or likely, to be infected with, pathogenic pneumococcal bacteria that produce CbpA, or the like, preferably *S. pneumoniae* bacteria (however a mouse

model can be utilized to simulate such a patient in some circumstances).

In addition to use as vaccines, the polypeptides of the present invention can be used as immunogens to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

The polynucleotides encoding the immunogenic polypeptides described above may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be, for example, a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The identification of multiple coil structures of alpha helical amino acid segments in the *S. pneumoniae* polypeptides according to the invention may be determined by the location of proline rich areas with respect to one another. Further the "X" area optionally located between two or more alpha-helical structures can play a part in the formation of a coil within a coil structure. Standard three-dimensional protein modeling may be utilized for determining the relative shape of such structures. An example of a computer program, the Paircoil Scoring Form Program ("PairCoil program"), useful for such three-dimensional protein modelling is described by Berger et al. in the Proc. Natl. Acad. of Sci. (USA), 92:8259-8263 (August 1995). The PairCoil program is described as a computer program that utilizes a mathematical algorithm to

predict locations of coiled-coil regions in amino acid sequences. A further example of such a computer program is described by Wolf et al., Protein Science 6:1179-1189 (June 1997) which is called the Multicoil Scoring Form Program ("Multicoil program"). The MultiCoil program is based on the PairCoil algorithm and is useful for locating dimeric and trimeric coiled coils .

In a preferred aspect, the invention provides for recombinant production of such polypeptides in a host bacterial cell other than a *S. pneumoniae* species host to avoid the inclusion of native surface-binding polypeptides that have a choline binding region. A preferred host is a species of such bacteria that can be cultured under conditions such that the polypeptide of the invention is secreted from the cell.

The present invention also relates to vectors which include polynucleotides encoding one or more of the polypeptides of the invention that include the highly conserved alpha-helical amino acid sequence in the absence of an area encoding a choline binding amino acid sequence, host cells which are genetically engineered with vectors of the invention and the production of such immunogenic polypeptides by recombinant techniques in an isolated and substantially immunogenically pure form.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors comprising a polynucleotide encoding a polypeptide comprising the highly conserved alpha-helical region but not having a choline binding region, or the like of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the polynucleotides which encode such polypeptides. The

culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampi-

cillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the proteins.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen, Inc.), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired

gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and TRP. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides

of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas,

Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, a french press, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art. However, preferred are host cells which secrete the polypeptide of the invention and permit recovery of the polypeptide from the culture media.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing

a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptides can be recovered and/or purified from recombinant cell cultures by well-known protein recovery and purification methods. Such methodology may include ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. In this respect, chaperones may be used in such a refolding procedure. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides that are useful as immunogens in the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Particularly preferred immunogens are truncated pneumococcal polypeptides that comprise a single highly conserved alpha helical area, but do not comprise a choline binding region or a HPS region. Therefore, antibodies against such polypeptides should bind to other pneumococcal bacterial species (in addition to the *S. pneumoniae* species from

which such polypeptides were derived) and vaccines against such *S. pneumoniae* should give protection against other pneumococcal bacterial infections.

Procedures for the isolation of the individually expressed alpha-helical containing polypeptides may be isolated by recombinant expression/isolation methods that are well-known in the art. Typical examples for such isolation may utilize an antibody to a conserved area of the protein or to a His tag or cleavable leader or tail that is expressing as part of the protein structure.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today

4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

In order to facilitate understanding of the above description and the examples which follow below, as well as the Figures included herewith, Table 1 below sets forth the bacterial source for the polypeptides of SEQ ID NOS:3-18 and the polynucleotides encoding them (SEQ ID NOS:20-35, respectively). The name and/or type of bacteria is specified and a credit or source is named. The sequences from such types of bacteria are for illustrative purposes only since by utilizing probes and/or primers as described herein other sequences of similar type may be readily obtained by utilizing only routine skill in the art.

TABLE 1

SEQ ID NO.	Type Of Pneumococcus	Source Credit or ATCC No.
3	1	ATCC 33400
4	2	SPATCC 11733
5	2	ATCC2 (catalog #6302)
6	4	ATCC4 (catalog #6304)
7	6B	ATCC 6B (catalog #6326)
8	18C	SPATCC 18C (ATCC catalog #10356)

TABLE 1
(Continued)

SEQ ID NO.	Type Of Pneumococcus	Source Credit or ATCC No.
9	4	Norway type 4; Nat'l. Inst. of Public Health, Norway Ingeborg Aagerge
10	noncapsulated	R6X; Rockefeller Univ., Rob Masure (from D39, type 2)
11	6B	SPB 105; Boston Univ., Steve Pelton
12	23F	SPB 328; Boston Univ., Steve Pelton
13	14	SPB 331; Boston Univ., Steve Pelton
14	23F	SPB 365; Boston Univ., Steve Pelton
15	9V	SPR 332; Rockefeller Univ., Rob Masure
16	6B	SPSJ 2p; St. Jude Children's Research Hospital, Pat Flynn (clinical isolate passaged 1x in mice for virulence)
17	14	SPSJ 9; St. Jude Children's Research Hospital, Pat Flynn (clinical isolate - nares, pneumonia)
18	19A	SPSJ 12; St. Jude Children's Research Hospital, Pat Flynn (clinical isolate)

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

Example 1

Generation of CbpA Truncate Protein Vectors

A. *Vector for Full Length CbpA (NR1XR2PC)*

A virulent serotype 4 *S. pneumoniae* strain, Norway 4 (obtained from I. Aaberge, National Instute of Public Health, Oslo, Norway) was used as a source of genomic DNA template for amplifying the polynucleotide encoding full-length CbPA. Full length CbpA was amplified with PCR primers SJ533 and SJ537 described below.

The degenerate forward primer SJ533 was designed based on the CbpA N-terminal sequence XENEG provided by H.R. Masure (St. Jude Childern's Research Hospital, Memphis, TN). The SJ533 primer = 5′ GGC GGA TCC ATG GA(A,G) AA(C,T) GA(A,G) GG 3′. It incorporates both BamHI and NcoI restriction sites and an ATG start codon.

The 3′ reverse primer SJ537 = 5′ GCC GTC GAC TTA GTT TAC CCA TTC ACC ATT GGC 3′

This primer incorporates a SalI restriction site for cloning purposes, and the natural stop codon from CbpA, and is based on type 4 and R6X sequence generated in-house.

PCR product generated from genomic DNA template with SJ533 and SJ537 was digested with BamHI and SalI, and cloned into the pQE30 expression vector (Qiagen, Inc.) digested with BamHI, XbaI, and SmaI. The type R6X template resulted in full-length vector PMI581 and the type 4 template DNA resulted in full-length vector PMI580.

B. *Vector for CbpA Truncate Protein (NR1XR2)*

The naturally occurring PvuII site in the end on the second amino repeat (nucleic acid 1228 of Type 4 sequence) was exploited to create a truncated version of

CbpA, containing only the amino terminus of the gene. To create the truncate clone, the full-length clone PMI580 (Type 4) or PMI581 (R6X) was digested with PvuII and XbaI, and the amino terminus along with a portion of the expression vector was isolated by size on an agarose gel. pQE30 was digested with XbaI and SmaI, and the band corresponding to the other half of the vector was also size selected on an agarose gel. The two halves were ligated and clones identified by restriction digest, then expressed. In this instance, the stop codon utilized is in the expression vector, so the protein expressed is larger than the predicted size due to additional amino acids at the 5′ and 3′ end of the cloning site.

C. *Vector for CbpA Truncate Protein (NR1X)*

A similar strategy was used to express only the first amino repeat of CbpA. Here the naturally occurring XmnI site between the two amino repeats (nucleic acid 856 of Type 4 sequence) was utilized. CbpA full-length clone PMI580 was digested with XmnI and AatII. Expression vector pQE30 was digested with AatII and SmaI. Once again, the two sized fragments were ligated, and clones were screened by restriction digest and expressed.

Example 2

Expression of CbpA Truncate Protein From Expression Vectors

All proteins are expressed from the vectors described in Examples 1A-1C in the Qia expressionist System (Qiagen) using the *E. coli* expression vector pQE30, and the amino terminus His6 tagged proteins are detected by western analysis using both anti-Histidine antibodies and gene specific antibodies.

The expressed CBP truncates were purified as follows.

A single colony was selected from plated bacteria

containing the recombinant plasmid and grown overnight at 37° in 6.0 ml LB buffer with 50 ug/ml Kanamycin and 100 ug/ml Ampicillin. This 6.0 ml culture was added to 1L LB with antibiotics at above concentrations. The culture was shaken at 37°C until A_{600} = ~0.400. 1M IPTG was added to the 1L culture to give a final concentration of 1mM. The culture was then shaken at 37°C for 3-4 h. The 1L culture was spun 15 min. in 250 ml conical tubes at 4000 rpm in a model J-6B centrifuge. The supernatant was discarded and the pellet stored at -20°C until use.

The 1L pellet was resuspended in 25 ml 50 mM NaH_2PO_4, 10mM Tris, 6M GuCl, 300mM NaCl, pH 8.0 (Buffer A). This mixture was then rotated at room temperature for 30 minutes. The mixture was then subjected to sonication (VibraCell Sonicator, Sonics and Materials, Inc., Danbury, CT) using the microtip, two times, for 30 sec., at 50% Duty Cycle and with the output setting at 7. The mixture was spun 5 min. at 10K in a JA20 rotor and the supernatant removed and discarded. The supernatant was loaded on a 10 ml Talon (Clonetech, Palo Alto, CA) resin column attached to a GradiFrac System (Pharmacia Biotech, Upsala, Sweden). The column was equilibrated with 100 ml Buffer A and washed with 200 ml of this buffer. A volume based pH gradient using 100% 50 mM NaH_2PO_4, 8M Urea, 20mM MES, pH 6.0 (Buffer B) as the final target buffer was run over a total volume of 100 ml. Protein eluted at ~ 30% Buffer B. Eluted peaks were collected and pooled.

For refolding, dialysis was carried out with a 2L volume of PBS at room temperature for approximately 3 hr. using dialysis tubing with a molecular weight cutoff of 14,000. The sample was then dialyzed overnight in 2L of PBS at 4°C. Additional buffer exchange was accomplished during the concentration of the protein using Centriprep-30 spin columns by adding PBS to the spun retentate and

respinning. The protein concentration was determined using the BCA protein assay and the purity visualized using a Coomassie stained 4-20% SDS-PAGE gel.

Example 3

Passive Protection with Anti-CbpA Truncate NR1XR2 Antisera

A. *Generation of Rabbit Immune Serum*

Rabbit immune serum against CbpA truncate was generated at Covance (Denver, PA). Following collection of preimmune serum, a New Zealand white rabbit (#ME101) was immunized with 250 µg CbpA truncate NR1XR2 (containing both alpha helix I and alpha helix II amino acid N-terminal repeats that are prepared from 483:58) in Complete Freund's Adjuvant. The rabbit was given a boost of 125 µg CbpA truncate in Incomplete Freund's Adjuvant on day 21 and bled on days 31 and 52.

B. *Passive Protection in Mice*

C3H/HeJ mice (5 mice/group) were passively immunized intraperitoneally with 100 µl of a 1:2 dilution of rabbit sera in sterile PBS (preimmune or day 31 immune sera). One hour after administration of serum, mice were challenged with 1600 cfu virulent serotype 6B *S. pneumoniae*, strain SP317 (obtained from H.R. Masure). Mice were monitored for 14 days for survival.

Eighty percent of the mice immunized with rabbit immune serum raised against CbpA truncate NR1XR2 protein survived the challenge for 14 days (Figure 2). All mice immunized with preimmune rabbit serum were dead by day 7.

C. *Passive Protection in Mice (Higher Challenge Dose)*

C3H/HeJ mice (10 mice/group) were passively immunized intraperitoneally with 100 µl of a 1:2 dilution of rabbit sera in sterile PBS (preimmune or day 52 immune sera). One hour after administration of serum, mice were challenged with 3450 cfu virulent serotype 6B *S. pneumoniae*, strain SP317. Mice were monitored for 10 days for survival.

One hundred percent of the mice immunized with rabbit immune serum raised against CbpA truncate NR1XR2 protein survived the challenge for ten days (Figure 3). Ninety percent of the mice immunized with preimmune rabbit serum were dead at day 10.

D. *Passive Protection in Mice (Against High Virulence)*

C3H/HeJ mice (10 mice/group) were passively immunized intraperitoneally with 100 µl of a 1:2 dilution of rabbit sera in sterile PBS (preimmune or day 52 immune sera). One hour after administration of serum, mice were challenged with 580 cfu virulent serotype 6B *S. pneumoniae*, strain SPSJ2 (provided by P. Flynn, St. Jude Children's Research Hospital, Memphis, TN). Mice were monitored for 10 days for survival.

Fifty percent of the mice immunized with rabbit immune serum raised against CbpA truncate NR1XR2 protein survived the challenge for 10 days (Figure 4). All of the mice immunized with preimmune rabbit serum were dead at day 8.

These data demonstrate that antibodies specific for CbpA are protective against systemic pneumococcal infection. The data further indicate that the choline-binding region is not necessary for protection, as antibody specific for truncated protein NR1XR2, lacking the choline-binding repeats, was sufficient for protection. In addition, serum directed against

recombinant CbpA protein based on a serotype 4 sequence, was still protective against challenge with two different strains of serotype 6B.

Example 4

<u>Active Protection with Anti-CbpA Truncates NR1X and NR1XR2</u>

A. *Active Protection With NR1X Truncate Vaccination*

C3H/HeJ mice (10/group) were immunized intraperitoneally with CbpA truncate protein NR1X (15μg in 50 μl PBS, plus 50 μl Complete Freund's Adjuvant). A group of 10 sham immunized mice received PBS and adjuvant. A second immunization was administered four weeks later, 15 μg protein i.p. with Incomplete Freund's Adjuvant (sham group received PBS plus IFA). Blood was drawn (retro-orbital bleed) at weeks 3, 6, and 9 for analysis of immune response. The ELISA end point anti-CbpA truncate titer of pooled sera from the 10 CbpA immunized mice at 9 weeks was 4,096,000. No antibody was detected in sera from sham immunized mice. Mice were challenged at week 10 with 560 CFU serotype 6B *S. pneumoniae* strain SPSJ2. Mice were monitored for 14 days for survival.

Eighty percent of the mice immunized with CbpA truncate protein NR1X survived the challenge for 14 Days (results shown in Figure 5). All sham immunized mice were dead by day 8.

B. *Active Protection With NR1XR2 Truncate Vaccination*

C3H/HeJ mice (10/group) were immunized intraperitoneally with CbpA truncate protein NR1XR2 (15μg in 50 μl PBS, plus 50 μl Complete Freund's Adjuvant). A group of 10 control immunized mice received pneumococcal recombinant protein SP90 and adjuvant. A second

immunization was administered four weeks later, 15µg protein i.p. with Incomplete Freund's Adjuvant. Blood was drawn (retro-orbital bleed) at weeks 3, 6, and 9 for analysis of immune response. The ELISA end point anti-CbpA truncate titer of pooled sera from the 10 CbpA immunized mice at 9 weeks was 4,096,000. Mice were challenged at week 10 with 680 CFU serotype 6B *S. pneumoniae* strain SPSJ2. Mice were monitored for 14 days for survival.

Fifty percent of the mice immunized with CbpA truncate protein NR1XR2 survived the challenge for 14 days (results shown in Figure 6). All control immunized mice were dead by day 9.

These data demonstrate that immunization with recombinant CbpA truncate proteins elicit production of specific antibodies capable of protecting against systemic pneumococcal infection and death. The data further indicate that the choline-binding region is not necessary for protection, as the immunogens were truncated proteins NR1X and NR1XR2. Additionally, the results suggest that a single amino terminal repeat may be sufficient to elicit a protective response. Cross protection is demonstrated as the recombinant pneumococcal protein was generated based on serotype 4 DNA sequence and protection was observed following challenge with a serotype 6B isolate.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

WHAT IS CLAIMED IS:

1. A vaccine against bacterial infections comprising an immunogen which is a polypeptide truncate of a pneumococcal surface-binding protein, analog or variant having a highly conserved immunogenic alpha-helical portion with respect to different types of pneumococcal bacteria, which polypeptide does not include a choline-binding portion.

2. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has at least 75 % identity with respect to the amino acid sequence of SEQ ID NO:1.

3. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has at least 85 % identity with respect to the amino acid sequence of SEQ ID NO:1.

4. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has at least 90 % identity with respect to the amino acid sequence of a member consisting of:

(a) the amino acid sequence of SEQ ID NO:1, and
(b) the amino acid sequence of SEQ ID NO:19.

5. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has at least 95 % identity with respect to the amino acid sequence of a member selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:1, and
(b) the amino acid sequence of SEQ ID NO:19.

6. A vaccine according to claim 1, wherein said vaccine is for preventing or treating otitis media, sepsis, meningitis and lobar pneumonia infections.

7. A vaccine according to claim 6, wherein said vaccine is for invasive infections.

8. A vaccine according to claim 6, wherein said vaccine is for otitis media infections caused by *S. pneumoniae*.

9. A vaccine according to claim 1, wherein said polypeptide truncate comprise an amino acid sequence which has at least 90 % identity with respect to a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18.

10. A vaccine according to claim 1, wherein said polypeptide truncate comprise an amino acid sequence which has at least 95 % identity with respect to a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18.

11. An antibody raised against an immunogen which is a polypeptide truncate of a pneumococcal surface-binding protein, analog or variant having a highly conserved immunogenic alpha-helical portion with respect to different types of pneumococcal bacteria, which polypeptide does not include a choline-binding portion.

12. An antibody according to claim 11, wherein the amino acid sequence of said alpha-helical portion has at least 85 % identity with respect to the amino acid sequence of SEQ ID NO:1.

13. An antibody according to claim 11, wherein the amino acid sequence of said alpha-helical portion has at least 90 % identity with respect to the amino acid sequence of a member
selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:1,

and

(b) the amino acid sequence of SEQ ID NO:19.

14. An antibody according to claim 11, wherein the amino acid sequence of said alpha-helical portion has at least 95 % identity with respect to the amino acid sequence of a member selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:1, and

(b) the amino acid sequence of SEQ ID NO:19.

15. An antibody according to claim 11, wherein said polypeptide truncate comprise an amino acid sequence which has at least 95 % identity with respect to a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18.

16. An antibody according to claim 11, wherein said antibody is an antibody that will detect *S. pneumoniae* infections.

17. An antibody according to claim 15, wherein said antibody is effective for the prevention and/or treatment of *S. pneumoniae* infections.

18. An antibody according to claim 15, wherein said antibody is effective for the prevention and/or treatment of pneumococcal infections caused by types 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F *S. pneumoniae* bacteria.

19. A method for preventing and/or treating pneumococcal infections in a host comprising immunizing said host with a member selected from the group consisting of:

(a) a vaccine according to claim 2, and

(b) at least one antibody raised against an immunogen which is a polypeptide truncate of a pneumococcal surface-binding protein, analog or variant

comprising an amino acid sequence that is has at least 90 % identity to the amino acid sequence of a member selected from the group consisting of SEQ ID NO:3 to 18, which polypeptide does not include a choline-binding portion.

20. A polypeptide comprising an amino acid sequence which has at least 90 % identity with respect to a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18.

21. An isolated polynucleotide comprising polynucleotide sequence having at least 90 % identity to a member selected from the group consisting of:

(a) a polynucleotide coding sequence encoding a polypeptide comprising a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18, and

(b) and the complement of (a).

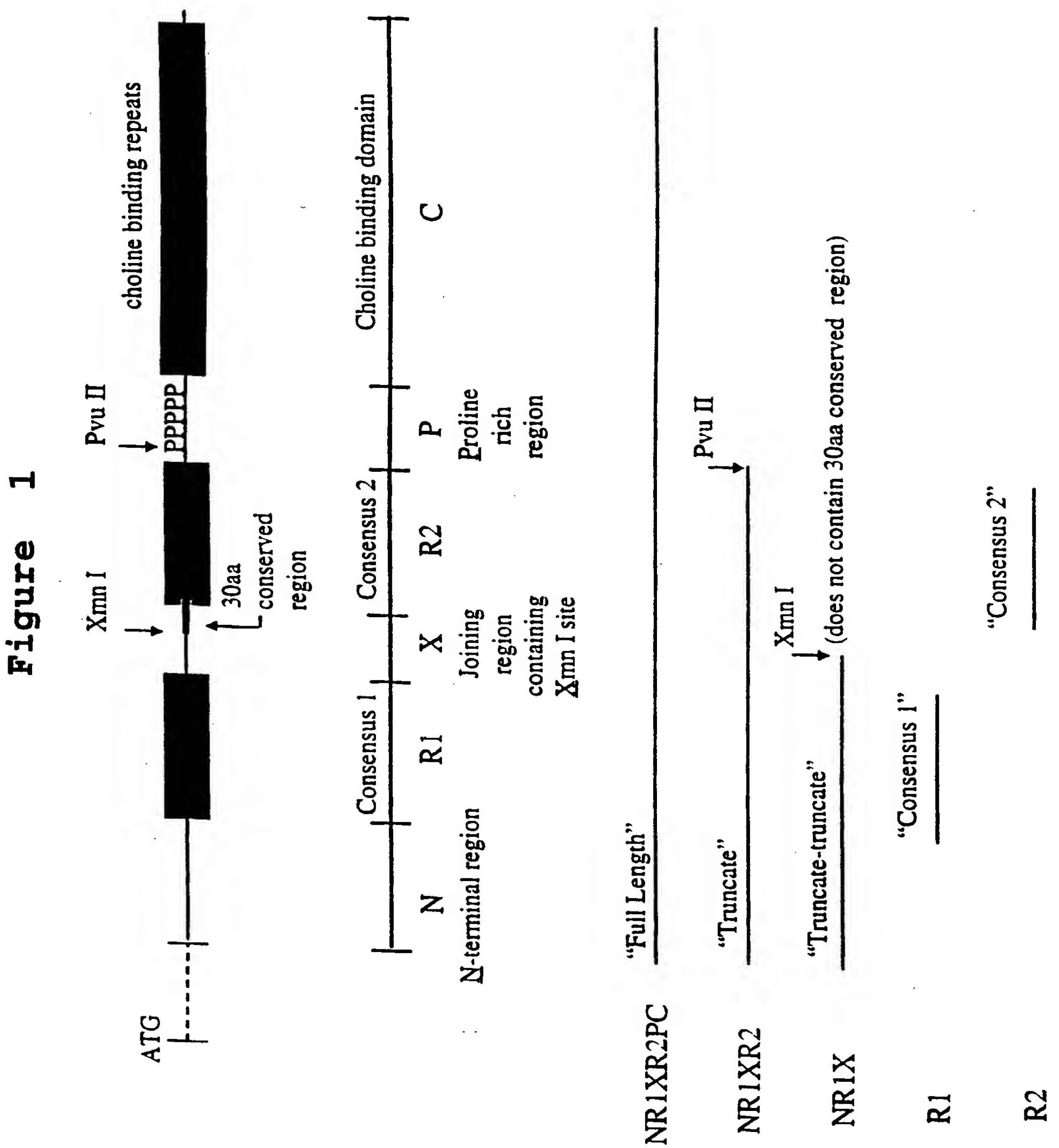

Figure 1

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Figure 2

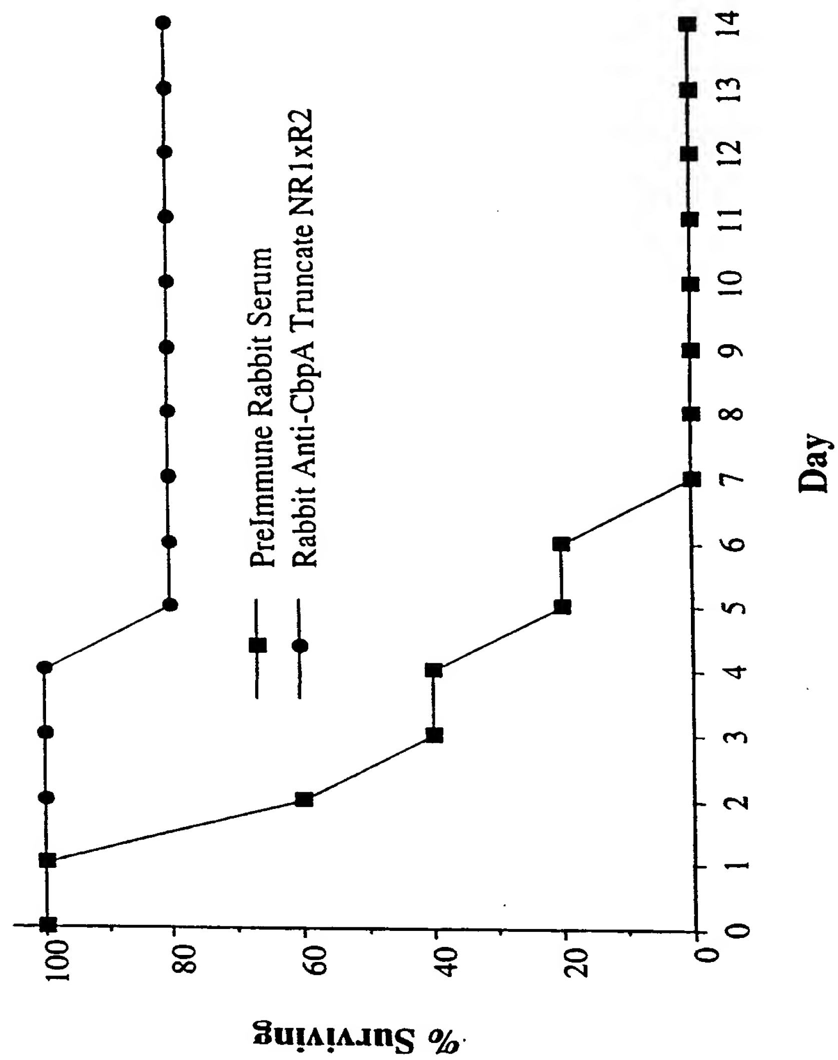

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Figure 3

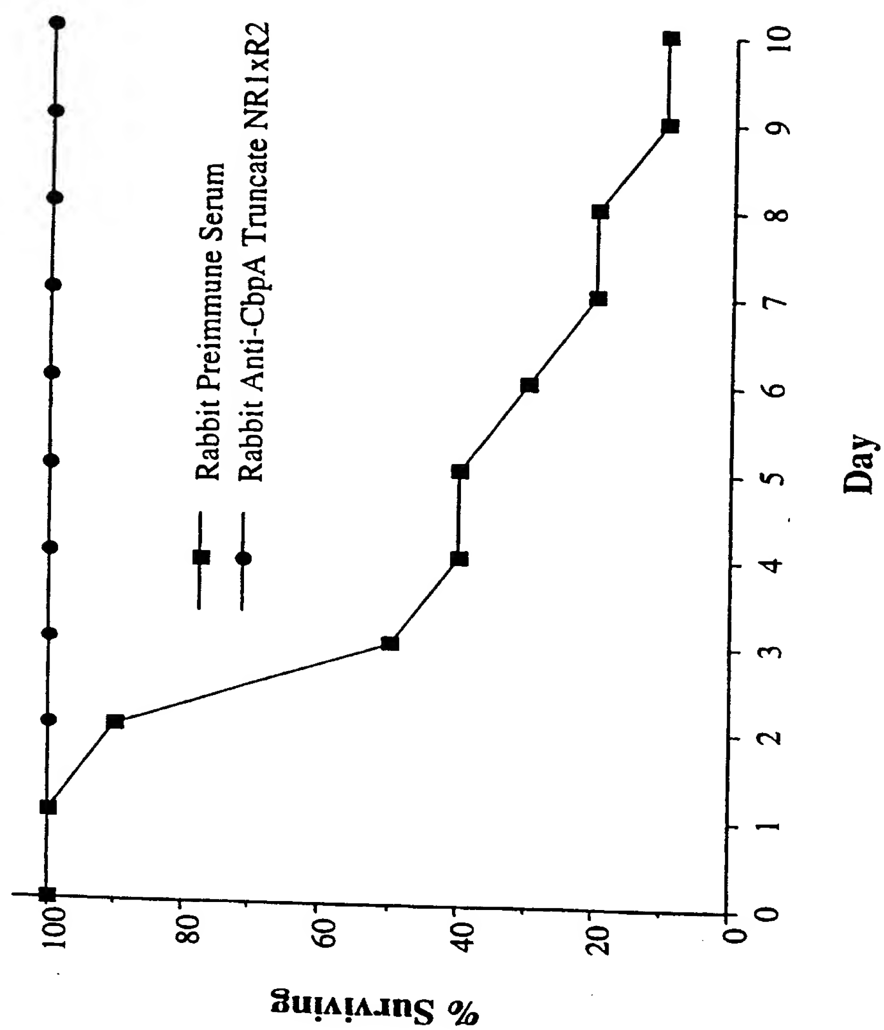

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Figure 4

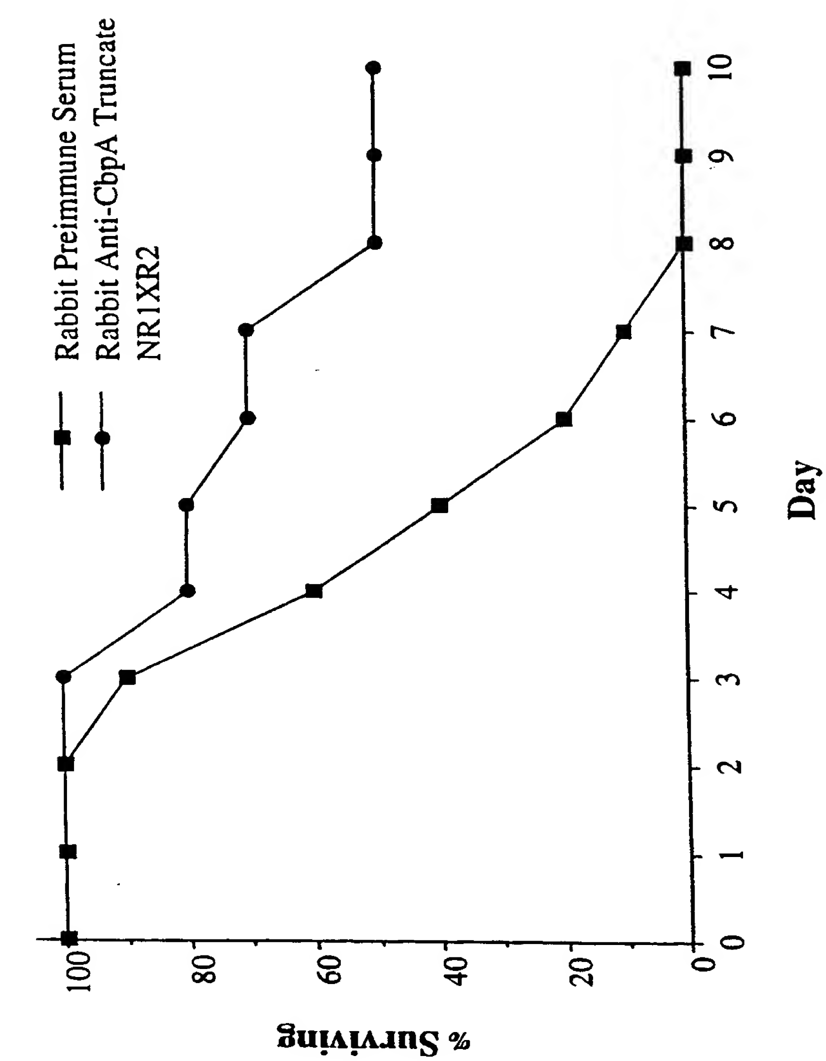

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Figure 5

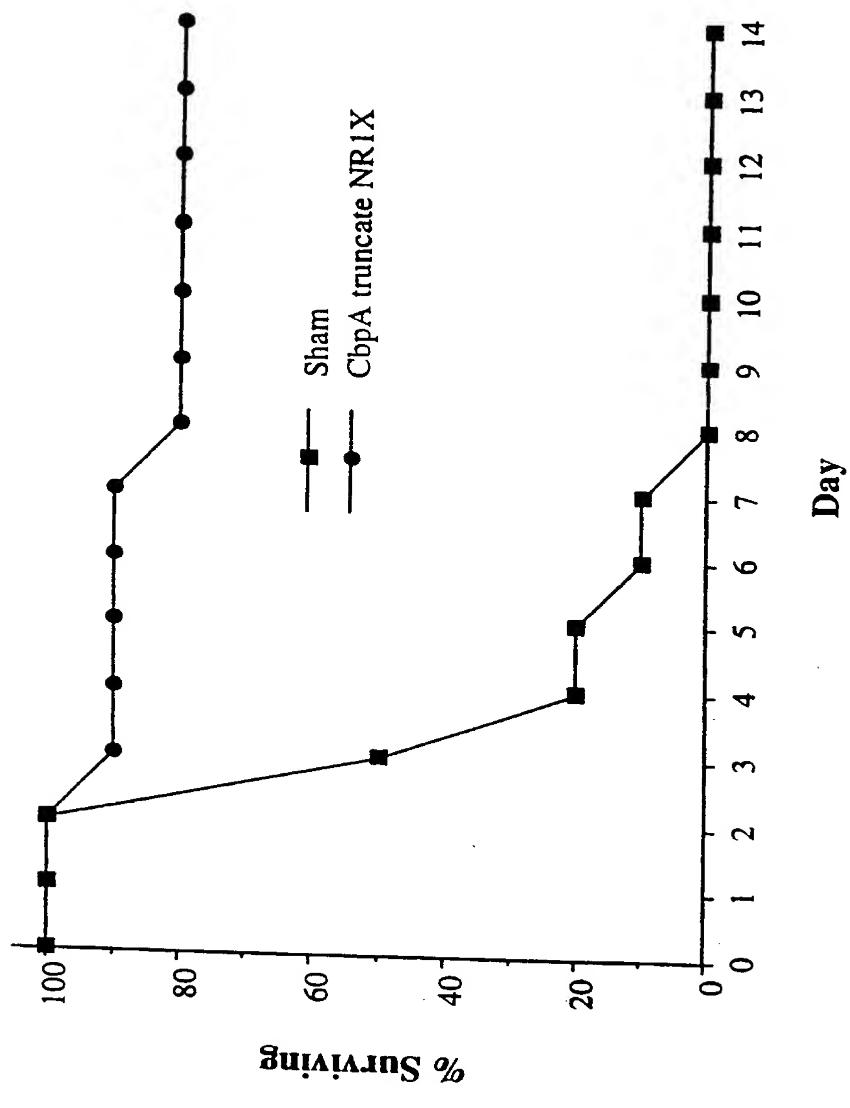

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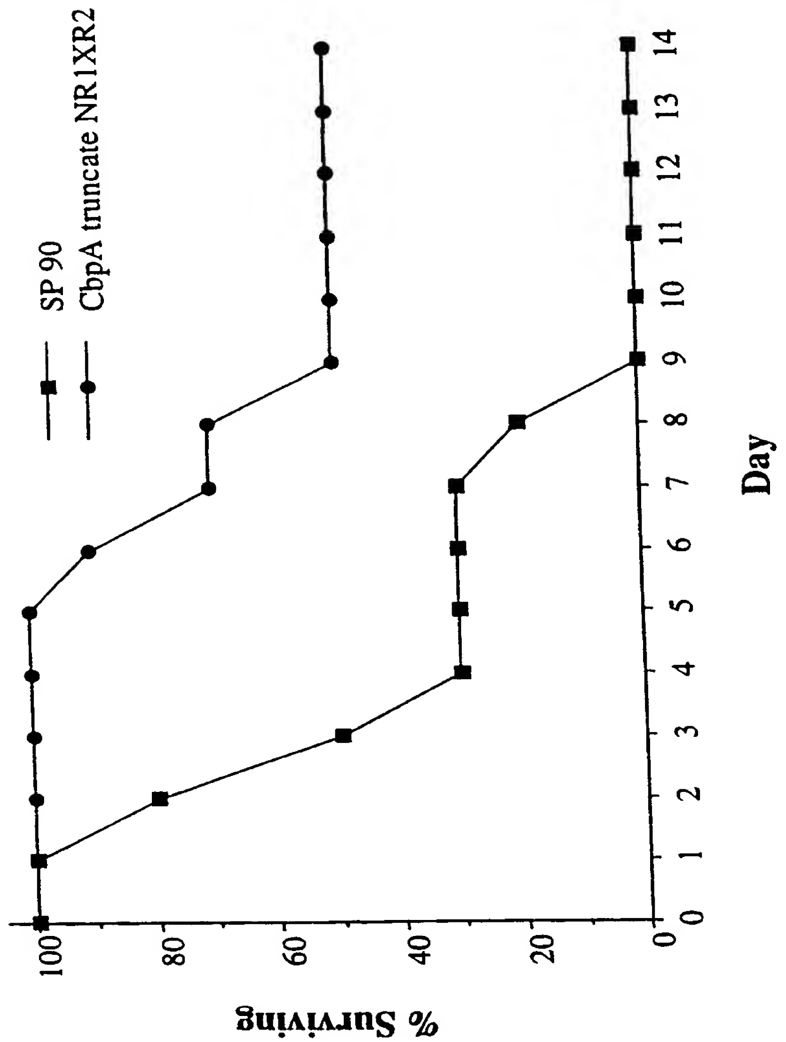
Figure 6



(Sheet 6 of 16)

Figure 7

```
                                  10        20        30        40        50        60        70
     TENE-GTTQVATSSNRAN--QTEH----------RKAAKQVVDEYIKKML-E-Q--LDRRKHTQNVALNIK    Majority
 1   TENE-GATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVEEYVKKIVGESYAKSTKKRHTITVALVNE   Norway4
 1   TEKE-VTTEVATSSNKANKSQTEH----------MKAAEQV-DEYINKMI---Q--LDKRKHTQNLALNIK    ATCC33400(1)
 1   TENE-GSTQAATSSNMAK---TEH----------RKAAKQVVDEYIEKMLREIQ--LDRRKHTQNVALNIK    ATCC11733(2)
 1   TEKE-VTTQVPTYSNMAK---TEH----------RKAAKQVVDEYIEKMLREIQ--LDRRKHTQNFAFNMK    ATCC2
 1   TENE-GATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVEEYVKKIVGESYAKSTKKRHTITVALVNE   ATCC4
 1   EGVRSGNNSTVTSSG-----QDIS----------KKYADE-VESHLQSILKDVNKNLKKVQHTQNADFNKK    ATCC6B
 1   EGVRSGNNSTVTSSG-----QDIS----------KKYADE-VESHLQSILKDVNKNLKKVQHTQNADFNKK    ATCC18C
 1   TENE-GSTQAATSSNMAK---TEH----------RKAAKQVVDEYIEKMLREIQ--LDRRKHTQNVALNIK    R6X(2)
 1   TEKE-VTTQVATSSNRANESQAGH----------RKAAEQE-DEYIKTMI---Q--LDRRKHTQNFALNIK    SPB105(6B)
 1   TEKE-VTTQVATSSNKANKSQTEH----------MKAAKQV-DEYIKKKL---Q--LDRRKHTQNVGLLTK    SPB328(23F)
 1   TEKE-VTTQVATSSNKANKSQTEH----------MKAAKQV-DEYIKKKL---Q--LDRRKHTQNVGLLTK    SPB331(14)
 1   TEKE-VTTQVATSSNRANKSQTEH----------MKAAKQV-DEYIKKKL---Q--LDRRKHTQNVGLLTK    SPB365(23F)
 1   EEVGGRNTPTVTSSG-----QDIS----------KKYADE-VESHLKKILSEIQTQLDRKRLTKTVALLNE    SPB609(6B)
 1   TENE-RTTQVPTSSNRGKP---ER----------RKAAEQE-DEYINKMI---Q--LDKRKHTQNLAFNIQ    SPR332(9V)
 1   TENE-GSTQAATSSNMAK---TEH----------RKAAKQVVDEYIEKMLREIQ--LDRRKHTQNVALNIK    SPSJ2(6B)p
 1   EGVRSENNPTVTSSG-----QDIS----------KKYADE-VKSHLEKILSEIQTNLDRSKHIKTVNLINK    SPSJ9(14)
 1   TENE-GTTQAPTSSNRGNESQAEH----------MKAAKQV-DEYIEKML---Q--LDRRKHTQNVGLLTR    SPSJ12(19A)

              80        90        100       110       120       130       140
     LSAIKTEYLRE---------LNVL--EEKSKAE-LPS---EIKA---KLDAAFEQFKKDTLK----TEPG    Majority
71   LNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAVSKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPG    Norway4
55   LSAIKTKYLRE---------LNVL--EEKSKKEELTS---KTKK---EIDAAFEQFNKDTLK-----P-G    ATCC33400(1)
56   LSAIKTKYLRE---------LNVL--EEKSKDE-LPS---EIKA---KLDAAFEKFKKDTLK------PG    ATCC11733(2)
56   LSAIKTEYLYG---------LK-----EKSEAE-LPS---EVKA---KLDAAFEQFKKDTLK------LG    ATCC2
71   LNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAVSKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPG    ATCC4
56   LSKIKTKYLYE---------LNVL--EEKSEAE-LTSKTKETKE---EITAAFEQFKKDTLS----TEPE    ATCC6B
56   LSKIKPKYLYE---------LKQL--EEKSEAE-LTSKPKN-KR---RVTAAFEQFKKDTLS----TEPE    ATCC18C
56   LSAIKTKYLRE---------LNVL--EEKSKDE-LPS---EIKA---KLDAAFEKFKKDTLK------PG    R6X(2)
55   LSRIKTEYLRK---------LNVL--EEKSKAE-LPS---ETKK---EIDAAFEQFKKDTNR----T--K    SPB105(6B)
55   LGVIKTEYLHG---------LSVS--KKKSEAE-LPS---EIKA---KLDAAFEQFKKDTLP----TEPG    SPB328(23F)
55   LGVIKTEYLHG---------LSVS--KKKSEAE-LPS---EIKA---KLDAAFEQFKKDTLP----TEPG    SPB331(14)
55   LGVIKTEYLHG---------LSVS--KKKSEAE-LPS---EIKA---KLDAAFEQFKKDTLP----TEPG    SPB365(23F)
56   LQNIKKTYLYN---------LNVL--KEKS--E-LPVKN---KS---KVRS                        SPB609(6B)
52   LSRIKTEYLNG---------L-----KEKSEAE-LPS---KIKA---ELDAAFKQFKKDTLP----TEPE    SPR332(9V)
56   LSAIKTKYLRE---------LNVL--EEKSKDE-LPS---EIKA---KLDAAFEKFKKDTLK------PG    SPSJ2(6B)p
56   LQDIKRTYLYE---------LNVL--EDKSKAE-LPSKI---KA---ELDAAFEQFKKDTLP----TEPG    SPSJ9(14)
55   LGAIKTEYLRG---------LSVS--KEKSTAE-LPS---EIKE---KLTAAFKQFKKDTLK----PE      SPSJ12(19A)
```

Decoration 'Decoration #1': Box residues that differ from the Consensus.

Decoration 'Decoration #3': Box residues that match the Consensus exactly.

(Sheet 7 of 16)

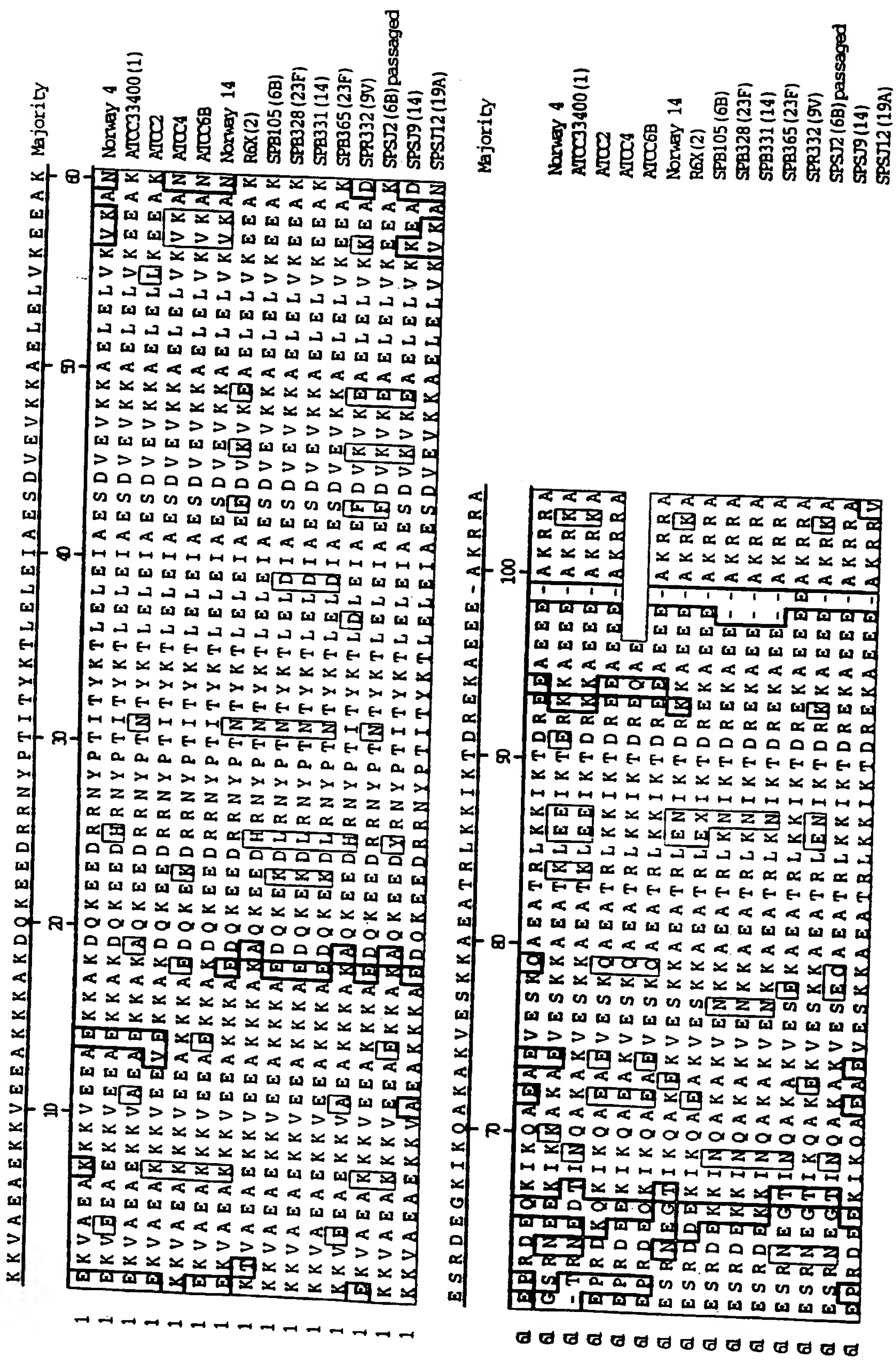
Figure 8

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1		30.7	31.7	28.6	100.0	19.8	19.0	31.7	33.0	36.3	36.3	37.3	30.2	32.3	31.7	23.3	37.0	1	Norway4
2			63.4	55.1	30.7	39.6	34.7	63.4	75.0	65.3	65.3	64.4	27.9	59.4	63.4	37.6	62.0	2	ATCC33400(1)
3				78.6	31.7	40.6	36.6	100.0	62.0	60.4	60.4	60.4	30.2	59.4	100.0	46.5	65.0	3	ATCC11733(2)
4					28.6	37.8	36.7	78.6	58.2	63.3	63.3	63.3	24.4	67.7	78.6	38.8	60.2	4	ATCC2
5						19.8	19.0	31.7	33.0	36.3	36.3	37.3	30.2	32.3	31.7	23.3	37.0	5	ATCC4
6							89.5	40.6	41.0	38.2	38.2	37.3	51.2	41.7	40.6	64.1	38.0	6	ATCC6B
7								36.6	35.0	35.3	35.3	34.3	48.8	38.5	36.6	60.2	34.0	7	ATCC18C
8									62.0	60.4	60.4	60.4	30.2	59.4	100.0	46.5	65.0	8	R6X(2)
9										70.0	70.0	71.0	27.9	65.6	62.0	42.0	67.0	9	SPB105(6B)
10											100.0	99.0	27.9	63.5	60.4	45.1	81.0	10	SPB328(23F)
11												99.0	27.9	63.5	60.4	45.1	81.0	11	SPB331(14)
12													27.9	64.6	60.4	45.1	82.0	12	SPB365(23F)
13														23.3	30.2	66.3	29.1	13	SPB609(6B)
14															59.4	42.7	63.5	14	SPR332(9V)
15																46.5	65.0	15	SPSJ2(6B)p
16																	41.0	16	SPSJ9(14)
17																		17	SPSJ12(19A)

(Sheet 8 of 16)

Figure 9



Decoration 'Decoration #2': Box residues that differ from the Consensus.

Decoration 'Decoration #3': Box residues that match the Consensus exactly.

(Sheet 9 of 16)

Figure 10

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1		80.8	77.9	98.1	92.7	100.0	78.8	82.7	79.8	79.8	79.8	76.0	78.8	83.7	91.3	1	Norway 4
2			86.5	78.8	76.0	80.8	82.7	83.7	78.8	78.8	78.8	76.9	82.7	77.9	79.8	2	ATCC33400(1)
3				76.0	74.0	77.9	84.6	82.7	79.8	79.8	79.8	78.8	84.6	80.8	77.9	3	ATCC2
4					90.6	98.1	76.9	81.7	77.9	77.9	77.9	74.0	76.9	81.7	89.4	4	ATCC4
5						92.7	79.2	83.3	84.4	84.4	84.4	76.0	79.2	81.2	93.8	5	ATCC6B
6							78.8	82.7	79.8	79.8	79.8	76.0	78.8	83.7	91.3	6	Norway 14
7								82.7	82.7	82.7	82.7	81.7	100.0	83.7	78.8	7	R6X(2)
8									86.5	86.5	86.5	80.8	82.7	81.7	85.6	8	SPB105(6B)
9										100.0	100.0	78.8	82.7	80.8	83.7	9	SPB328(23F)
10											100.0	78.8	82.7	80.8	83.7	10	SPB331(14)
11												78.8	82.7	80.8	83.7	11	SPB365(23F)
12													81.7	91.3	79.8	12	SPR332(9V)
13														83.7	78.8	13	SPSJ2(6B)passaged
14															79.8	14	SPSJ9(14)
15																15	SPSJ12(19A)

(Sheet 10 of 16)

Figure 11

```
                                  10        20        30        40        50        60
   DAKL-EA------TSEQDK----PKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPS   Majority
1  DAK------------EQGK----PKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPS   Norway4
1  -----EAEE---------EVKNKLKKRTKRGAEGEPATPDKKENDAKSSDSSVVKKSSK-   ATCC33400(1)
1  E-------------AEED-KVKDKLKRRTKRAVPGEPATPDKKENDAKSSDSSVGEETLPS   ATCC2
1  DAK------------EQGK----PKGRPKRGVPGELATPDKKENDAKSSDSSVGEETLPS   ATCC4
1  ATRLENIKTDREQAEEEAKVKDEPKKRTKRGVLGEPATPDKKENDAKSSDSSVGEETLPS   ATCC6B
1  DAK------------EQGK----PKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPS   Norway14
1  DAKLKEA---NVATSDQGK----PKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPS   R6X(2)
1  EAKLKEAVEKNVATSEQDK----PKGRRKRGVPGEQATPDKKENDAKSSDSSVGEEALPS   SPB105(6B)
1  DAKLQEA---NVATSEQDK----SKRRAKREVXGELATPDKKENDAKSSDSSVGEETLTS   SPB328(23F)
1  DAKLQEA---NVATSEQDK----SKRRAKREVFGELATPDKKENDAKSSDSSVGEETLTS   SPB331(14)
1  DAKLQEA---NVATSEQDK----SKRRAKREVLGELATPDKKENDAKSSDSSVGEETLTS   SPB365(23F)
1  DAKLKEAVENNAATSEQGE----PKRRVKRGVFGEPATPDKKENDAKSSDSSVGEETLPS   SPB609(6B)
1  DAK------------EQDES-KRRKSRGKRGALGEQATPDKKENDAKSSDSSVGEETLPS   SPR332(9V)
1  DAKLKEA---NVATSDQGK----PKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPS   SPSJ2(6B)passaged
1  DAK------------EQDES-KRRKSRVKRGDFGEPATPDKKENDAKSSDSSVGEETLPS   SPSJ9(14)
1  DAK------------EQDESSKRRKSRXKRGDVGEQATPDKKENDAKSSDSSVGEETLPS   SPSJ12(19A)

          70
   PSLKPE-------   Majority
45 PSLKPE          Norway4
46 PILKSEKKVAEAE   ATCC33400(1)
48 PSLKSG          ATCC2
45 PSLKPE          ATCC4
61 PSLKPE          ATCC6B
45 PSLKPE          Norway14
54 SSLKSG          R6X(2)
57 PSLKPE          SPB105(6B)
54 PSLKPE          SPB328(23F)
54 PSLKPE          SPB331(14)
54 PSLKPE          SPB365(23F)
57 PSLKPE          SPB609(6B)
48 PSLKPG          SPR332(9V)
54 SSLKSG          SPSJ2(6B)passaged
48 PSLKPG          SPSJ9(14)
49 PSLKPG          SPSJ12(19A)
```

Decoration 'Decoration #1': Box residues that differ from the Consensus.

Decoration 'Decoration #2': Box residues that match the Consensus exactly.

(Sheet 11 of 16)

Figure 12

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1		52.0	76.0	98.0	82.0	100.0	86.0	86.0	82.0	82.0	82.0	84.0	76.0	86.0	76.0	76.0	1	Norway4
2			58.5	52.0	55.2	52.0	43.1	44.8	43.1	44.8	43.1	48.3	49.1	43.1	50.9	46.3	2	ATCC33400(1)
3				76.0	81.1	76.0	71.7	71.7	69.8	69.8	69.8	69.8	69.8	71.7	71.7	69.8	3	ATCC2
4					82.0	98.0	84.0	86.0	80.0	80.0	80.0	84.0	76.0	84.0	76.0	76.0	4	ATCC4
5						82.0	62.7	64.5	64.4	64.4	66.1	67.7	71.7	62.7	71.7	70.4	5	ATCC6B
6							86.0	86.0	82.0	82.0	82.0	84.0	76.0	86.0	76.0	76.0	6	Norway14
7								79.7	81.4	81.4	81.4	78.0	66.0	100.0	66.0	66.7	7	R6X(2)
8									78.0	78.0	78.0	83.9	69.8	79.7	67.9	66.7	8	SPB105(6B)
9										98.3	98.3	78.0	67.9	81.4	67.9	64.8	9	SPB328(23F)
10											98.3	79.7	67.9	81.4	69.8	64.8	10	SPB331(14)
11												78.0	69.8	81.4	67.9	64.8	11	SPB365(23F)
12													69.8	78.0	75.5	66.7	12	SPB609(6B)
13														66.0	92.5	88.7	13	SPR332(9V)
14															66.0	66.7	14	SPSJ2(6B)passaged
15																88.7	15	SPSJ9(14)
16																	16	SPSJ12(19A)

(Sheet 12 of 16)

Figure 13

Start	Sequence	Strain
	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	Majority
	10 20 30 40 50 60	
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	Norway 4
1	KKVAEAEKKVAEAEKKAKDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	ATCC33400(1)
1	EKVAEAKKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVKVKEAELELVKEEAK	ATCC11733(2)
1	KKVAEAEKKVAEAEKKAKDQKEEDRRNYPTNTYKTLELEIAEEDVKVKEAELELVKEEAK	ATCC2
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAK	ATCC4
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	ATCC6B
1	KKVAEAKKKVEEAKKKAEDQKEKDRRNYPTITYKTLELEIAESDVEVKKAELELVKEEAK	ATCC18C-3
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	Norway14
1	KKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	R6X(2)
1	KKVAEAEKKVAEAEKKAKAQKEEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAK	SPB105(6B)
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVKVKEAELELVKEEAK	SPB328(23F)
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	SPB331(14)
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	SPB365(23F)
1	KKVAEAEKKVEEADKKAKAQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	SPR332(9V)
1	KKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLELEIAESDVKVKEAELELVKEEAK	SPSJ2(6B)passaged
1	KKVAEAEKKVEEAEKKAKDQKEEDHRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAK	SPSJ9(14)
1	KKVAEAEKKVEEADKKAKAQKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVKEEAK	SPSJ12(19A)
1	KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK	Consensus A

Start	Sequence	Strain
	ESRNEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKA	Majority
	70 80 90 100	
61	EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKA	Norway 4
61	EPQNEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKA	ATCC33400(1)
61	ESRNEGTIKQAKEKVESKKAEATRLEKIKTDRKKAEE--AKRKV	ATCC11733(2)
61	GSRNEEKINQAKAEVESKKAEATRLENIKTDRKKAEEE-AKRKA	ATCC2
61	EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKA	ATCC4
61	EPRNEEKVKQAKAEVESKQAEATRLENIKTDRKKAEEE-AKRKA	ATCC6B
61	EPRNEEKVKQAKAEVESKQAEATRLEKIKTDRKKAEEE-AKRKA	ATCC18C-3
61	EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKA	Norway14
61	EPRDEEKIKQAKAKVESKKAEATRLENIKTDRKKAEEE-AKRKA	R6X(2)
61	ESRNEEKVNQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKA	SPB105(6B)
61	ESRNEEKIKQVKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKA	SPB328(23F)
61	ESRNEEKIKQVKAKVESKKAEATRLENIKTDRKRAEEEEAKRRA	SPB331(14)
61	ESRNEEKIKQVKAKVESKKAEATRLENIKTDRKKAEEEEAKRRA	SPB365(23F)
61	ESRNEEKIKQAKAKVESKKAEATRLENIKTDRKRAEEEEAKRRA	SPR332(9V)
61	EPRDEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKA	SPSJ2(6B)passaged
61	GSRNEEKVKQAKAEVESKKAEATRLENIKTDRKKAEEE-AKRKA	SPSJ9(14)
61	EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKA	SPSJ12(19A)
61	ESRNEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKA	Consensus A

Decoration 'Decoration 1': Box residues that differ from the Consensus.

(Sheet 13 of 16)

Figure 14

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1		88.2	88.3	90.3	100.0	98.1	96.1	100.0	91.3	91.3	90.3	91.3	90.3	92.2	91.3	94.2	97.1	97.1	1	Norway 4
2			84.3	90.2	88.2	86.3	84.3	88.2	91.2	91.2	86.3	87.3	86.3	91.2	91.2	86.3	88.2	89.2	2	ATCC33400(1)
3				86.4	88.3	88.3	86.4	88.3	89.3	87.4	86.4	87.4	86.4	90.3	89.3	84.5	85.4	91.3	3	ATCC11733(2)
4					90.3	88.3	86.4	90.3	93.2	95.1	84.5	85.4	84.5	93.2	93.2	92.2	90.3	91.3	4	ATCC2
5						98.1	96.1	100.0	91.3	91.3	90.3	91.3	90.3	92.2	91.3	94.2	97.1	97.1	5	ATCC4
6							96.1	98.1	91.3	89.3	90.3	91.3	90.3	90.3	91.3	92.2	95.1	95.1	6	ATCC6B
7								96.1	87.4	87.4	86.4	87.4	86.4	88.3	87.4	92.2	93.2	93.2	7	ATCC18C-3
8									91.3	91.3	90.3	91.3	90.3	92.2	91.3	94.2	97.1	97.1	8	Norway14
9										92.2	87.4	88.3	87.4	94.2	100.0	89.3	91.3	92.2	9	R6X(2)
10											85.4	86.4	85.4	96.1	92.2	91.3	93.2	92.2	10	SPB105(6B)
11												99.0	100.0	88.3	87.4	86.4	87.4	93.2	11	SPB328(23F)
12													99.0	89.3	88.3	87.4	88.3	94.2	12	SPB331(14)
13														88.3	87.4	86.4	87.4	93.2	13	SPB365(23F)
14															94.2	91.3	95.1	95.1	14	SPR332(9V)
15																89.3	91.3	92.2	15	SPSJ2(6B)passaged
16																	94.2	93.2	16	SPSJ9(14)
17																		94.2	17	SPSJ12(19A)
18																			18	Consensus A

(Sheet 14 of 16)

Figure 15

```
                                   10        20        30        40        50        60
     KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  Majority
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  Norway4
 1   KKVAEAEKKVAEAEKKAKDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  ATCC33400(1)
 1   EKVAEAKKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVKVKEAELELVKEEAK  ATCC11733(2)
 1   KKVAEAEKKVAEAEKKAKDQKEEDRRNYPTNTYKTLELEIAEFDVKVKEAELELVKEEAK  ATCC2
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAK  ATCC4
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  ATCC6B
 1   KKVAEAKKKVEEAKKKAEDQKEKDRRNYPTITYKTLELEIAESDVEVKKAELELVKEEAK  ATCC18C-3
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  Norway14
 1   KKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESDVEVKKAELELVKEEAK  R6X(2)
 1   KKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAK  SPB105(6B)
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAK  SPB328(23F)
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  SPB331(14)
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  SPB365(23F)
 1   KKVAEAEKKVEEADKKAKAQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  SPR332(9V)
 1   KKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAK  SPSJ2(6B)passaged
 1   KKVAEAEKKVEEAEKKAKDQKEEDHRNYPTITYKTLELEIAESDVKVKEAELELVKEEAK  SPSJ9(14)
 1   KKVAEAEKKVEEADKKAKAQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  SPSJ12(19A)
 1   KKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAK  Consensus B

              70        80        90        100       110
     EPRNEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  Majority
61   EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  Norway4
61   EPQNEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKVAEEDKVKEKPA  ATCC33400(1)
61   ESRNEGTIKQAKEKVESKKAEATRLENIKTDRKKAEE--AKRKAAEEDKVKEKPA  ATCC11733(2)
61   GSRNEEKINQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  ATCC2
61   EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  ATCC4
61   EPRNEEKVKQAKAEVESKQAEATRLENIKTDRKKAEEE-AKRKAAEEDKVKEKPA  ATCC6B
61   EPRNEEKVKQAKAEVESKQAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  ATCC18C-3
61   EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  Norway14
61   EPRDEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  R6X(2)
61   EPRDEEKIKQAKAKVESKKAEATRLENIKTDRKKAEEE-AKRKAAEEDKVKEKPA  SPB105(6B)
61   ESRNEEKIKQVKAKVESKKAEATRLENIKTDRKKAEEE-AKRKAAEEDKVKEKPA  SPB328(23F)
61   ESRNEEKIKQVKAKVESKKAEATRLENIKTDRKRAEEEEAKRRAAEEDKVKEKPA  SPB331(14)
61   ESRNEEKIKQVKAKVESKKAEATRLENIKTDRKKAEEEEAKRRAAEEDKVKEKPA  SPB365(23F)
61   ESRNEEKIKQAKAKVESKKAEATRLENIKTDRKRAEEEEAKRRAAEEDKVKEKPA  SPR332(9V)
61   EPRDEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  SPSJ2(6B)passaged
61   GSRNEEKVKQAKAEVESKKAEATRLENIKTDRKKAEEE-AKRKAAEEDKVKEKPA  SPSJ9(14)
61   EPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  SPSJ12(19A)
61   EPRNEEKIKQAKAKVESKKAEATRLEKIKTDRKKAEEE-AKRKAAEEDKVKEKPA  Consensus B
```

Decoration 'Decoration #1': Box residues that differ from the Consensus.

Decoration '': Box residues that match the Consensus exactly.

(Sheet 15 of 16)

Figure 16

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1		89.4	89.5	91.2	100.0	98.2	96.5	100.0	92.1	92.1	91.2	92.1	91.2	93.0	92.1	94.7	97.4	98.2	1	Norway4
2			85.8	91.2	89.4	87.6	85.8	89.4	92.0	92.0	86.7	87.6	86.7	92.0	92.0	87.6	89.4	91.2	2	ATCC33400(1)
3				87.7	89.5	89.5	87.7	89.5	90.4	90.4	87.7	88.6	87.7	91.2	90.4	86.0	86.8	91.2	3	ATCC11733(2)
4					91.2	89.5	87.7	91.2	93.9	93.9	86.0	86.8	86.0	93.9	93.9	93.0	91.2	91.2	4	ATCC2
5						98.2	96.5	100.0	92.1	92.1	91.2	92.1	91.2	93.0	92.1	94.7	97.4	98.2	5	ATCC4
6							96.5	98.2	92.1	92.1	91.2	92.1	91.2	91.2	92.1	93.0	95.6	96.5	6	ATCC6B
7								96.5	88.6	88.6	87.7	88.6	87.7	89.5	88.6	93.0	93.9	94.7	7	ATCC18C-3
8									92.1	92.1	91.2	92.1	91.2	93.0	92.1	94.7	97.4	98.2	8	Norway14
9										100.0	88.6	89.5	88.6	94.7	100.0	90.4	92.1	93.9	9	R6X(2)
10											88.6	89.5	88.6	94.7	100.0	90.4	92.1	93.9	10	SPB105(6B)
11												99.1	100.0	89.5	88.6	87.7	88.6	93.0	11	SPB328(23F)
12													99.1	90.4	89.5	88.6	89.5	93.9	12	SPB331(14)
13														89.5	88.6	87.7	88.6	93.0	13	SPB365(23F)
14															94.7	92.1	95.6	94.7	14	SPR332(9V)
15																90.4	92.1	93.9	15	SPSJ2(6B)passaged
16																	94.7	93.0	16	SPSJ9(14)
17																		95.6	17	SPSJ12(19A)
18																			18	Consensus B

(Sheet 16 of 16)

SEQUENCE LISTING

<110> Wizemann, Theresa M.
Koenig, Scott
Johnson, Leslie S

<120> Derivatives of Choline Binding Proteins for Vaccines

<130> 469201-364

<140>
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<150> US 60/085,743
<151> 1998-05-15

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20 25 30

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35 40 45

Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser Arg Asn
50 55 60

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1 5 10 15

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20 25 30

Glu Leu Glu Tyr Glu Val Gln Arg Leu Glu Lys Glu Leu Lys Glu Ile
35 40 45

Asp Glu Ser Asp Ser Glu Asp Tyr Leu Lys Glu Gly Leu Arg Ala Pro
50 55 60

Leu Gln Ser Lys Leu Asp Thr Lys Lys Ala Lys Leu Ser Lys Leu Glu
65 70 75 80

Glu Leu Ser Asp Lys Ile Asp Glu Leu Asp Ala Glu Ile Ala Lys Leu
85 90 95

Glu Val Gln Leu Lys Asp Ala Glu Gly Asn Asn Asn Val Glu Ala Tyr
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20 25 30

Tyr Ile Asn Lys Met Ile Gln Leu Asp Lys Arg Lys His Thr Gln Asn
35 40 45

Leu Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu Arg
50 55 60

Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Lys Glu Glu Leu Thr Ser
65 70 75 80

Lys Thr Lys Lys Glu Ile Asp Ala Ala Phe Glu Gln Phe Asn Lys Asp
85 90 95

Thr Leu Lys Pro Gly Glu Lys Val Glu Glu Ala Glu Lys Lys Val Glu
100 105 110

Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp His Arg Asn
115 120 125

Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser
130 135 140

Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala
145 150 155 160

Lys Gly Ser Arg Asn Glu Glu Lys Ile Lys Lys Ala Lys Ala Glu Val
165 170 175

Glu Ser Lys Lys Ala Glu Ala Thr Lys Leu Glu Glu Ile Lys Thr Glu
180 185 190

Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Glu Ala Glu Glu
195 200 205

Glu Val Lys Asn Lys Leu Lys Lys Arg Thr Lys Arg Gly Ala Phe Gly
210 215 220

Glu Pro Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser Ser Asp
225 230 235 240

Ser Ser Val Val Lys Lys Ser Ser Lys Pro Ile Leu Lys Ser Glu Lys
245 250 255

Lys Val Ala Glu Ala Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val
260 265 270

Ala Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp Arg Arg
275 280 285

Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu
290 295 300

Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu
305 310 315 320

Ala Lys Glu Pro Gln Asn Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys
325 330 335

Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr
340 345 350

Asp Arg Lys Lys Ala Glu Glu Ala Lys Arg Lys Val Ala Glu Glu Asp
355 360 365

Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro
370 375 380

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20 25 30

Glu Lys Met Leu Arg Glu Ile Gln Leu Asp Arg Arg Lys His Thr Gln
35 40 45

Asn Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu
50 55 60

Arg Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Asp Glu Leu Pro Ser
65 70 75 80

Glu Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Lys Phe Lys Lys Asp
85 90 95

Thr Leu Lys Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu
100 105 110

Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn
115 120 125

Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe
130 135 140

Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala
145 150 155 160

Lys Glu Ser Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val
165 170 175

Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp
180 185 190

Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp
195 200 205

Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Thr
210 215 220

Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro
225 230 235 240

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Streptococcus pneumoniae

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20 25 30

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35 40 45

Asn Phe Ala Phe Asn Met Lys Leu Ser Ala Ile Lys Thr Glu Tyr Leu
50 55 60

Tyr Gly Leu Lys Glu Lys Ser Glu Ala Glu Leu Pro Ser Glu Val Lys
65 70 75 80

Ala Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Leu Lys
85 90 95

Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Ala Glu Ala Glu
100 105 110

Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr
115 120 125

Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu
130 135 140

Val Lys Lys Ala Glu Leu Glu Leu Leu Lys Glu Glu Ala Lys Thr Arg
145 150 155 160

Asn Glu Asp Thr Ile Asn Gln Ala Lys Ala Lys Val Glu Ser Lys Lys
165 170 175

Ala Glu Ala Thr Leu Lys Glu Glu Ile Lys Thr Asp Arg Lys Lys Ala
180 185 190

Glu Glu Glu Ala Lys Arg Lys Ala Glu Ala Glu Glu Asp Lys Val Lys
195 200 205

Asp Lys Leu Lys Arg Arg Thr Lys Arg Ala Val Pro Gly Glu Pro Ala
210 215 220

Thr Phe Phe Lys Lys Glu Asn Asp Ala Lys Ser Ser Asp Ser Ser Val
225 230 235 240

Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys Ser Gly Lys Lys Val
245 250 255

Ala Glu Ala Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Ala Lys Asp
260 265 270

Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr Thr Lys Thr
275 280 285

Leu Asp Leu Glu Ile Ala Glu Ser Asp Val Lys Val Lys Glu Ala Glu
290 295 300

Leu Glu Leu Val Lys Glu Glu Ala Lys Gly Ser Arg Asn Glu Glu Lys
305 310 315 320

Ile Asn Gln Ala Lys Ala Glu Val Glu Ser Lys Lys Ala Glu Ala Thr
325 330 335

Arg Leu Glu Lys Thr Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala
340 345 350

Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu
355 360 365

Gln Pro Gln Pro Ala Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu
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20 25 30

Glu Arg Asp Lys Ala Arg Lys Glu Val Glu Glu Tyr Val Lys Lys Ile
35 40 45

Val Gly Glu Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr
50 55 60

Val Ala Leu Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn
65 70 75 80

Lys Ile Val Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met
85 90 95

Glu Ser Arg Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp
100 105 110

Ser Ser Ser Ser Ser Ser Ser Asp Ser Ser Thr Lys Pro Glu Ala Ser
115 120 125

Asp Thr Ala Lys Pro Asn Lys Pro Thr Glu Pro Gly Glu Lys Val Ala
130 135 140

Glu Ala Lys Lys Lys Val Glu Glu Val Glu Lys Lys Ala Lys Asp Gln
145 150 155 160

Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu
165 170 175

Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu
180 185 190

Glu Leu Val Lys Val Lys Ala Asn Glu Pro Arg Asp Lys Gln Lys Ile
195 200 205

Lys Gln Ala Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg
210 215 220

Leu Lys Lys Ile Lys Thr Asp Arg Glu Glu Ala Glu Glu Glu Ala Lys
225 230 235 240

Arg Arg Ala Asp Ala Lys Glu Gln Gly Lys Pro Lys Gly Arg Pro Lys
245 250 255

Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp
260 265 270

Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro
275 280 285

Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu
290 295 300

Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn
305 310 315 320

Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser
325 330 335

Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala
340 345 350

Lys Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val
355 360 365

Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp
370 375 380

Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp
385 390 395 400

Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro
405 410 415

Lys Thr Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu
420 425 430

Gln Pro Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
435 440 445

<210> 7
<211> 428
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 7

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Glu Gly Val Arg Ser Gly Asn Asn Ser Thr Val Thr Ser Ser Gly Gln
 1               5                  10                  15

Asp Ile Ser Lys Lys Tyr Ala Asp Glu Val Glu Ser His Leu Gln Ser
            20                  25                  30

Ile Leu Lys Asp Val Asn Lys Asn Leu Lys Lys Val Gln His Thr Gln
        35                  40                  45

Asn Ala Asp Phe Asn Lys Lys Leu Ser Lys Ile Lys Thr Lys Tyr Leu
    50                  55                  60

Tyr Glu Leu Asn Val Leu Glu Glu Lys Ser Glu Ala Glu Leu Thr Ser
65                  70                  75                  80

Lys Thr Lys Glu Thr Lys Glu Glu Leu Thr Ala Ala Phe Glu Gln Phe
                85                  90                  95

Lys Lys Asp Thr Leu Ser Thr Glu Pro Glu Lys Lys Val Ala Glu Ala
            100                 105                 110

Lys Lys Lys Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu
        115                 120                 125

Lys Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu
    130                 135                 140

Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu
145                 150                 155                 160

Val Lys Val Lys Ala Asn Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln
                165                 170                 175

Ala Glu Ala Lys Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu Lys
            180                 185                 190

Lys Ile Lys Thr Asp Arg Glu Gln Ala Glu Ala Thr Arg Leu Glu Asn
        195                 200                 205

Ile Lys Thr Asp Arg Glu Gln Ala Glu Glu Glu Ala Lys Val Lys Asp
    210                 215                 220

Glu Pro Lys Lys Arg Thr Lys Arg Gly Val Leu Gly Glu Pro Ala Thr
225                 230                 235                 240

Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly
                245                 250                 255

Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala
            260                 265                 270

Glu Ala Glu Lys Lys Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln
        275                 280                 285

Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu
    290                 295                 300

Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu
305                 310                 315                 320

Glu Leu Val Lys Glu Glu Ala Lys Glu Pro Arg Asn Glu Glu Lys Val
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325 330 335

Lys Gln Ala Lys Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg
340 345 350

Leu Glu Asn Ile Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys
355 360 365

Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln
370 375 380

Pro Gln Pro Ala Pro Ala Pro Gln Pro Glu Lys Pro Ala Pro Lys Asp
385 390 395 400

Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro
405 410 415

Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
420 425

<210> 8
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 8
Glu Gly Val Arg Ser Gly Asn Asn Ser Thr Val Thr Ser Ser Gly Gln
1 5 10 15

Asp Ile Ser Lys Lys Tyr Ala Asp Glu Val Glu Ser His Leu Gln Ser
20 25 30

Ile Leu Lys Asp Val Asn Lys Asn Leu Lys Lys Val Gln His Thr Gln
35 40 45

Asn Ala Asp Phe Asn Lys Lys Leu Ser Lys Ile Lys Pro Lys Tyr Leu
50 55 60

Tyr Glu Leu Lys Cys Leu Glu Glu Lys Ser Glu Ala Glu Leu Thr Ser
65 70 75 80

Lys Pro Lys Asn Lys Arg Arg Val Thr Ala Ala Phe Glu Gln Phe Lys
85 90 95

Lys Asp Thr Leu Ser Thr Glu Pro Glu Lys Lys Val Ala Glu Ala Lys
100 105 110

Lys Lys Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys
115 120 125

Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu
130 135 140

Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val
145 150 155 160

Lys Glu Glu Ala Lys Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala

165 170 175

Lys Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu Glu Lys
180 185 190

Ile Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala
195 200 205

Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala
210 215

<210> 9
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 9
Thr Glu Asn Glu Gly Ala Thr Gln Val Pro Thr Ser Ser Asn Arg Ala
1 5 10 15

Asn Glu Ser Gln Ala Glu Gln Gly Glu Gln Pro Lys Lys Leu Asp Ser
20 25 30

Glu Arg Asp Lys Ala Arg Lys Glu Val Glu Glu Tyr Val Lys Lys Ile
35 40 45

Val Gly Glu Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr
50 55 60

Val Ala Leu Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn
65 70 75 80

Lys Ile Val Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met
85 90 95

Glu Ser Arg Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp
100 105 110

Ser Ser Ser Ser Ser Ser Ser Asp Ser Ser Thr Lys Pro Glu Ala Ser
115 120 125

Asp Thr Ala Lys Pro Asn Lys Pro Thr Glu Pro Gly Glu Lys Val Ala
130 135 140

Glu Ala Lys Lys Lys Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln
145 150 155 160

Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu
165 170 175

Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu
180 185 190

Glu Leu Val Lys Val Lys Ala Asn Glu Pro Arg Asp Glu Gln Lys Ile
195 200 205

Lys Gln Ala Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg

210 215 220

Leu Lys Lys Ile Lys Thr Asp Arg Glu Glu Ala Glu Glu Glu Ala Lys
225 230 235 240

Arg Arg Ala Asp Ala Lys Glu Gln Gly Lys Pro Lys Gly Arg Ala Lys
245 250 255

Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp
260 265 270

Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro
275 280 285

Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu
290 295 300

Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn
305 310 315 320

Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser
325 330 335

Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala
340 345 350

Lys Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val
355 360 365

Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp
370 375 380

Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp
385 390 395 400

Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro
405 410 415

Lys Ala Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu
420 425 430

Gln Pro Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
435 440 445

<210> 10
<211> 414
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 10
Thr Glu Asn Glu Gly Ser Thr Gln Ala Ala Thr Ser Ser Asn Met Ala
1 5 10 15

Lys Thr Glu His Arg Lys Ala Ala Lys Gln Val Val Asp Glu Tyr Ile
20 25 30

Glu Lys Met Leu Arg Glu Ile Gln Leu Asp Arg Arg Lys His Thr Gln

35 40 45

Asn Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu
50 55 60

Arg Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Asp Glu Leu Pro Ser
65 70 75 80

Glu Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Lys Phe Lys Lys Asp
85 90 95

Thr Leu Lys Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu
100 105 110

Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn
115 120 125

Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe
130 135 140

Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala
145 150 155 160

Lys Glu Ser Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val
165 170 175

Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp
180 185 190

Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Asp Ala Lys Leu
195 200 205

Lys Glu Ala Asn Val Ala Thr Ser Asp Gln Gly Lys Pro Lys Gly Arg
210 215 220

Ala Lys Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
225 230 235 240

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro
245 250 255

Ser Ser Ser Leu Lys Ser Gly Lys Lys Val Ala Glu Ala Glu Lys Lys
260 265 270

Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp Arg
275 280 285

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Asp Leu Glu Ile Ala
290 295 300

Glu Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu
305 310 315 320

Glu Ala Lys Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Lys Ala
325 330 335

Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
340 345 350

Thr Asp Arg Asp Asp Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu
355 360 365

Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro
370 375 380

Ala Thr Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu
385 390 395 400

Gln Pro Lys Ala Glu Lys Thr Asp Asp Gln Gln Ala Glu Glu
405 410

<210> 11
<211> 425
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 11
Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Arg Ala
1 5 10 15

Asn Glu Ser Gln Ala Gly His Arg Lys Ala Ala Glu Gln Phe Asp Glu
20 25 30

Tyr Ile Lys Thr Met Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Phe Ala Leu Asn Ile Lys Leu Ser Arg Ile Lys Thr Glu Tyr Leu Arg
50 55 60

Lys Leu Asn Val Leu Glu Glu Lys Ser Lys Ala Glu Leu Pro Ser Glu
65 70 75 80

Thr Lys Lys Glu Ile Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
85 90 95

Asn Arg Thr Lys Lys Thr Val Ala Glu Ala Glu Lys Lys Val Glu Glu
100 105 110

Ala Lys Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp His Arg Asn Tyr
115 120 125

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
130 135 140

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
145 150 155 160

Glu Ser Arg Asp Asp Glu Lys Ile Lys Gln Ala Glu Ala Lys Val Glu
165 170 175

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
180 185 190

Glu Lys Ala Glu Glu Glu Ala Lys Arg Arg Ala Glu Ala Lys Leu Lys
195 200 205

Glu Ala Val Glu Lys Asn Val Ala Thr Ser Glu Gln Asp Lys Pro Lys
210 215 220

Gly Arg Arg Lys Arg Gly Val Pro Gly Glu Gln Ala Thr Pro Asp Lys
225 230 235 240

Lys Glu Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Ala
245 250 255

Leu Pro Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu
260 265 270

Lys Lys Val Ala Glu Ala Glu Lys Lys Ala Lys Ala Gln Lys Glu Glu
275 280 285

Asp Arg Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu
290 295 300

Ile Ala Glu Ser Asp Val Lys Val Lys Glu Ser Glu Leu Glu Leu Val
305 310 315 320

Lys Glu Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Val Asn Gln Ala
325 330 335

Lys Ala Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys
340 345 350

Ile Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala
355 360 365

Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro
370 375 380

Ala Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro
385 390 395 400

Ala Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys Ala Glu
405 410 415

Lys Thr Asp Asp Gln Gln Ala Glu Glu
420 425

<210> 12
<211> 426
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 12
Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Lys Ala
1 5 10 15

Asn Lys Ser Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
20 25 30

Tyr Ile Lys Lys Lys Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Gly Leu Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His
50 55 60

Gly Leu Ser Val Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
85 90 95

Leu Pro Thr Glu Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val
100 105 110

Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg
115 120 125

Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu
130 135 140

Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu
145 150 155 160

Ala Lys Glu Ser Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys
165 170 175

Val Glu Asn Lys Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr
180 185 190

Asp Arg Glu Lys Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu
195 200 205

Gln Glu Ala Asn Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg
210 215 220

Ala Lys Arg Glu Val Leu Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
225 230 235 240

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr
245 250 255

Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
260 265 270

Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg
275 280 285

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
290 295 300

Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu
305 310 315 320

Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala
325 330 335

Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
340 345 350

Thr Asp Arg Lys Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala
355 360 365

Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala
370 375 380

Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala
385 390 395 400

Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala
405 410 415

Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
420 425

<210> 13
<211> 425
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 13
Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Lys Ala
1 5 10 15

Asn Lys Ser Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
20 25 30

Tyr Ile Lys Lys Lys Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Gly Leu Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His
50 55 60

Gly Leu Ser Val Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
85 90 95

Leu Pro Thr Glu Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val
100 105 110

Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg
115 120 125

Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu
130 135 140

Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu
145 150 155 160

Ala Lys Glu Ser Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys
165 170 175

Val Glu Asn Lys Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr
180 185 190

Asp Arg Glu Lys Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu
195 200 205

Gln Glu Ala Asn Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg
210 215 220

Ala Lys Arg Glu Val Phe Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
225 230 235 240

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr
245 250 255

Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
260 265 270

Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg
275 280 285

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
290 295 300

Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu
305 310 315 320

Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala
325 330 335

Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
340 345 350

Thr Asp Arg Lys Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala
355 360 365

Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala
370 375 380

Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala
385 390 395 400

Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala
405 410 415

Glu Lys Pro Ala Asp Gln Gln Ala Glu
420 425

<210> 14
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 14
Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Arg Ala
1 5 10 15

Asn Lys Ser Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
20 25 30

Tyr Ile Lys Lys Lys Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Gly Leu Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His
50 55 60

Gly Leu Ser Val Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
85 90 95

Leu Pro Thr Glu Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val
100 105 110

Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg
115 120 125

Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu
130 135 140

Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu
145 150 155 160

Ala Lys Glu Ser Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys
165 170 175

Val Glu Asn Lys Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr
180 185 190

Asp Arg Glu Lys Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu
195 200 205

Gln Glu Ala Asn Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg
210 215 220

Ala Lys Arg Glu Val Leu Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
225 230 235 240

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr
245 250 255

Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
260 265 270

Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg
275 280 285

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
290 295 300

Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu
305 310 315 320

Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala
325 330 335

Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
340 345 350

Thr Asp Arg Lys Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala
355 360 365

Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala
370 375 380

Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala
385 390 395 400

Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala

405 410 415

Glu Lys Pro Ala Asp Gln Gln Ala
420

<210> 15
<211> 419
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 15
Thr Glu Asn Glu Arg Thr Thr Gln Val Pro Thr Ser Ser Asn Arg Gly
1 5 10 15

Lys Pro Glu Arg Arg Lys Ala Ala Glu Gln Phe Asp Glu Tyr Ile Asn
20 25 30

Lys Met Ile Gln Leu Asp Lys Arg Lys His Thr Gln Asn Leu Ala Phe
35 40 45

Asn Ile Gln Leu Ser Arg Ile Lys Thr Glu Tyr Leu Asn Gly Leu Lys
50 55 60

Glu Lys Ser Glu Ala Glu Leu Pro Ser Lys Ile Lys Ala Glu Leu Asp
65 70 75 80

Ala Ala Phe Lys Gln Phe Lys Lys Asp Thr Leu Pro Thr Glu Pro Glu
85 90 95

Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Glu Lys Lys
100 105 110

Val Ala Glu Ala Lys Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp His
115 120 125

Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Asp Leu Glu Ile Ala
130 135 140

Glu Phe Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Lys
145 150 155 160

Glu Ala Asp Glu Ser Arg Asn Glu Gly Thr Ile Asn Gln Ala Lys Ala
165 170 175

Lys Val Glu Ser Glu Lys Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys
180 185 190

Thr Asp Arg Glu Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Asp
195 200 205

Ala Lys Glu Gln Asp Glu Ser Lys Arg Arg Lys Ser Arg Gly Lys Arg
210 215 220

Gly Ala Leu Gly Glu Gln Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala
225 230 235 240

Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser

245 250 255

Leu Lys Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu
260 265 270

Ala Asp Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp Arg Arg Asn Tyr
275 280 285

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
290 295 300

Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
305 310 315 320

Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys Val Glu
325 330 335

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg
340 345 350

Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys
355 360 365

Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Gln
370 375 380

Pro Glu Lys Pro Ala Glu Glu Pro Glu Asn Pro Val Pro Ala Pro Lys
385 390 395 400

Pro Glu Asn Pro Ala Glu Gln Pro Lys Ala Glu Lys Pro Ala Asp Gln
405 410 415

Gln Ala Glu

<210> 16
<211> 414
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 16
Thr Glu Asn Glu Gly Ser Thr Gln Ala Ala Thr Ser Ser Asn Met Ala
1 5 10 15

Lys Thr Glu His Arg Lys Ala Ala Lys Gln Val Val Asp Glu Tyr Ile
20 25 30

Glu Lys Met Leu Arg Glu Ile Gln Leu Asp Arg Arg Lys His Thr Gln
35 40 45

Asn Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu
50 55 60

Arg Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Asp Glu Leu Pro Ser
65 70 75 80

Glu Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Lys Glu Lys Lys Asp

85 90 95

Thr Leu Lys Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu
100 105 110

Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn
115 120 125

Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe
130 135 140

Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala
145 150 155 160

Lys Glu Ser Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val
165 170 175

Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp
180 185 190

Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Asp Ala Lys Leu
195 200 205

Lys Glu Ala Asn Val Ala Thr Ser Asp Gln Gly Lys Pro Lys Gly Arg
210 215 220

Ala Lys Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
225 230 235 240

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro
245 250 255

Ser Ser Ser Leu Lys Ser Gly Lys Lys Val Ala Glu Ala Glu Lys Lys
260 265 270

Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp Arg
275 280 285

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Asp Leu Glu Ile Ala
290 295 300

Glu Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu
305 310 315 320

Glu Ala Lys Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Lys Ala
325 330 335

Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
340 345 350

Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu
355 360 365

Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro
370 375 380

Ala Thr Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu
385 390 395 400

Gln Pro Lys Ala Glu Lys Thr Asp Asp Gln Gln Ala Glu Glu
405 410

<210> 17
<211> 412
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 17
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Glu Gly Val Arg Ser Glu Asn Asn Pro Thr Val Thr Ser Ser Gly Gln
 1               5                  10                  15

Asp Ile Ser Lys Lys Tyr Ala Asp Glu Val Lys Ser His Leu Glu Lys
            20                  25                  30

Ile Leu Ser Glu Ile Gln Thr Asn Leu Asp Arg Ser Lys His Ile Lys
        35                  40                  45

Thr Val Asn Leu Ile Asn Lys Leu Gln Asp Ile Lys Arg Thr Tyr Leu
    50                  55                  60

Tyr Glu Leu Asn Val Leu Glu Asp Lys Ser Lys Ala Glu Leu Pro Ser
65                  70                  75                  80

Lys Ile Lys Ala Glu Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp
                85                  90                  95

Thr Leu Pro Thr Glu Pro Gly Lys Lys Val Ala Glu Ala Lys Lys Lys
            100                 105                 110

Val Glu Glu Ala Glu Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp Tyr
        115                 120                 125

Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
    130                 135                 140

Glu Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Lys
145                 150                 155                 160

Glu Ala Asp Glu Ser Arg Asn Glu Gly Thr Ile Asn Gln Ala Lys Ala
                165                 170                 175

Lys Val Glu Ser Glu Gln Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys
            180                 185                 190

Thr Asp Arg Glu Lys Ala Glu Glu Glu Ala Lys Arg Arg Ala Asp Ala
        195                 200                 205

Lys Glu Gln Asp Glu Ser Lys Arg Arg Lys Ser Arg Val Lys Arg Gly
    210                 215                 220

Asp Phe Gly Glu Pro Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys
225                 230                 235                 240

Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu
                245                 250                 255

Lys Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala
            260                 265                 270
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Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp His Arg Asn Tyr Pro
275 280 285

Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val
290 295 300

Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Gly
305 310 315 320

Ser Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser
325 330 335

Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys
340 345 350

Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val
355 360 365

Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Gln Pro
370 375 380

Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro
385 390 395 400

Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
405 410

<210> 18
<211> 406
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of Streptococcus pneumoniae

<400> 18
Thr Glu Asn Glu Gly Thr Thr Gln Ala Pro Thr Ser Ser Asn Arg Gly
1 5 10 15

Asn Glu Ser Gln Ala Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
20 25 30

Tyr Ile Glu Lys Met Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Gly Leu Leu Thr Lys Leu Gly Ala Ile Lys Thr Glu Tyr Leu Arg
50 55 60

Gly Leu Ser Val Ser Lys Glu Lys Ser Thr Ala Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Glu Lys Leu Thr Ala Ala Phe Lys Gln Phe Lys Lys Asp Thr
85 90 95

Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Ala Glu
100 105 110

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
115 120 125

Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
130 135 140

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Val Lys Ala Asn
145 150 155 160

Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Glu Ala Glu Val Glu
165 170 175

Ser Lys Lys Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg
180 185 190

Glu Lys Ala Glu Glu Glu Ala Lys Arg Arg Val Asp Ala Lys Glu Gln
195 200 205

Asp Glu Ser Ser Lys Arg Arg Lys Ser Arg Val Lys Arg Gly Asp Leu
210 215 220

Gly Glu Gln Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser Ser
225 230 235 240

Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys Pro
245 250 255

Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Asp Lys
260 265 270

Lys Ala Lys Ala Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn
275 280 285

Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val
290 295 300

Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Pro Arg
305 310 315 320

Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser Lys Lys
325 330 335

Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys Ala
340 345 350

Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu
355 360 365

Lys Pro Ala Glu Gln Pro Lys Pro Ala Pro Ala Pro Gln Pro Glu Lys
370 375 380

Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro Lys Ala Glu Lys
385 390 395 400

Pro Ala Asp Gln Gln Ala
405

<210> 19
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from the genome of

Streptococcus pneumoniae

<400> 19
Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Lys Lys Lys
1   5   10   15

Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr
            20   25   30

Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys
        35   40   45

Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser Arg Asn
    50   55   60

Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys Val Glu Ser Lys Lys Ala
65   70   75   80

Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys Ala Glu
                85   90   95

Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys
            100   105   110

Pro Ala

<210> 20
<211> 1295
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA derived from the genome S. pneumoniae

<400> 20
acagagaagg aggtaactac cccagtagcc acttcttcta ataaggcaaa taaaagtcag 60
acagaacata tgaaagctgc tgaacaagtc gatgaatata taaacaaaat gatccaatta 120
gataaaagaa aacataccca aaatctcgcc ttaaacataa agttgagcgc aattaaaacg 180
aagtatttgc gtgaattaaa tgttttagaa gagaagtcga aaaaagaaga gttgacgtca 240
aaaacaaaaa aagagataga cgcagctttt gagcagttta acaaagatac attgaaacca 300
ggagaaaagg ttgaagaagc tgagaagaag gttgaagaag ctgagaaaaa agccaaggat 360
caaaaagaag aagatcaccg taactaccca accattactt acaaaacgct tgaacttgaa 420
attgctgagt ccgatgtgga agttaaaaaa gcggagcttg aactagtaaa agaggaagct 480
aagggatctc gaaacgagga aaaaattaag aaagcaaaag cggaagttga gagtaaaaaa 540
gctgaggcta caaagttaga agaaatcaag acagaacgta aaaaagcaga agaagaagct 600
aaacgaaaag cagaagcaga agaagaagtt aaaaataaac taaagaagcg gacaaaacga 660
ggagcttttg gagagccagc aacacctgat aaaaaagaaa atgatgcgaa gtcttcagat 720
tctagcgtgg tgaagaaatc ttccaagccc atcctgaaat cagaaaaaaa agtagcagaa 780
gctgagaaga aggttgcaga agctgagaag aaggttgcag aagctgagaa aaaagccaag 840
gatcaaaaag aagaagatcg ccgtaactac ccaaccaata cttacaaaac gcttgaactt 900
gaaattgctg agtccgatgt gaaagttaaa gaagcggagc ttgaactagt aaaagaggaa 960
gctaaggaac ctcaaaacga ggaaaaaatt aagcaagcaa aagcgaaagt tgagagtaaa 1020
aaagctgagg ctacaaggtt agaaaaaatc aagacagatc gtaaaaaagc agaagaagct 1080
aaacgaaaag tagcagaaga agataaagtt aaagaaaaac cagctgaaca accacaacca 1140
gctcctgcac caaaaccagc gccggctcct caaccagaaa aaccagctga acaaccaaaa 1200
gcagaaaaac cagctgatca acaagctgaa gaagactatg ctcgtagatc agaagaagaa 1260
tataacccgc ttgacttaac agcaccggca aaagc 1295

<210> 21
<211> 755

<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA from Streptococcus pneumoniae

<400> 21

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acagagaacg agggaagtac ccaagcagcc acttcttcta atatggcaaa gacagaacat      60
aggaaagctg ctaaacaagt cgtcgatgaa tatatagaaa aaatgttgag ggagattcaa     120
ctagatagaa gaaaacatac ccaaaatgtc gccttaaaca taaagttgag cgcaattaaa     180
acgaagtatt tgcgtgaatt aaatgtttta gaagagaagt cgaaagatga gttgccgtca     240
gaaataaaag caaagttaga cgcagctttt gagaagttta aaaaagatac attgaaacca     300
ggagaaaagg tagcagaagc taagaagaag gttgaagaag ctaagaaaaa agccgaggat     360
caaaaagaag aagatcgtcg taactaccca accaatactt acaaaacgct tgaacttgaa     420
attgctgagt tcgatgtgaa agttaaagaa gcggagcttg aactagtaaa agaggaagct     480
aaagaatctc gaaacgaggg cacaattaag caagcaaaag agaaagttga gagtaaaaaa     540
gctgaggcta caaggttaga aaacatcaag acagatcgta aaaaagcaga agaagaagct     600
aaacgaaaag cagcagaaga agataaagtt aaagaaaaac cagctgaaca accacaacca     660
gcgccggcta ctcaaccaga aaaaccagct ccaaaaccag agaagccagc tgaacaacca     720
aaagcagaaa aaacagatga tcaacaagct gaaga                                755
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<210> 22
<211> 1239
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA from Streptococcus pneumoniae

<400> 22

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acagagaagg aggtaactac ccaagtaccc acttattcta atatggcaaa gacagaacat      60
aggaaagctg ctaaacaagt cgtcgatgaa tatatagaaa aaatgttgag ggagattcaa     120
ttagatagaa gaaaacatac ccaaaatttc gccttcaaca tgaagttgag cgcaattaaa     180
acggagtatt tgtatggatt aaaagagaag tcggaagctg agttgccgtc agaagtaaaa     240
gcaaagttag acgcagcttt tgagcagttt aaaaaagata cattgaaact aggagaaaag     300
gtagcagaag ctgagaagaa ggttgcagaa gctgagaaaa aagccaaggc tcaaaaagaa     360
gaagatcgcc gtaactaccc aaccaatact tacaaaacgc ttgaacttga aattgctgag     420
tccgatgtgg aagttaaaaa agcggagctt gaactattga aagaggaagc taaaactcga     480
aacgaggaca caattaacca agcaaaagcg aaagttgaga gtaaaaaagc tgaggctaca     540
aagttagaag aaatcaagac agatcgtaaa aaagcagaag aagaagctaa acgaaaagca     600
gaagcagaag aagataaagt taaagataaa ctaaagaggc ggacaaaacg agcagttcct     660
ggagagccag caacacctga taaaaaagaa aatgatgcga agtcttcaga ttctagcgta     720
ggtgaagaaa ctcttccaag cccatccctg aaatcaggaa aaaaggtagc agaagctgag     780
aagaaggttg cagaagctga gaaaaaagcc aaggatcaaa aagaagaaga tcgccgtaac     840
tacccaacca atacttacaa aacgcttgac cttgaaattg ctgagtccga tgtgaaagtt     900
aaagaagcgg agcttgaact agtaaaagag gaagctaagg gatctcgaaa cgaggaaaaa     960
attaaccaag caaaagcgga agttgagagt aaaaaagctg aggctacaag gctagaaaaa    1020
atcaagacag atcgtaaaaa agcagaagaa gaagctaaac gaaaagcagc agaagaagat    1080
aaagttaaag aaaaaccagc tgaacaacca caaccagcgc cggctcctca accagaaaaa    1140
ccaactgaag agcctgagaa tccagctcca gctccaaaac cagagaagcc agctgaacaa    1200
ccaaaagcag aaaaaacaga tgatcaacaa gctgaagaa                           1239
```

<210> 23
<211> 1338
<212> DNA
<213> Artificial Sequence

<220>
<213> Description: cDNA from Streptococcus pneumoniae

<400> 23

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acagagaacg agggagctac ccaagtaccc acttcttcta atagggcaaa tgaaagtcag     60
gcagaacaag gagaacaacc taaaaaactc gattcagaac gagataaggc aaggaaagag    120
gtcgaggaat atgtaaaaaa aatagtgggt gagagctatg caaatcaac taaaaagcga    180
catacaatta ctgtagctct agttaacgag ttgaacaaca ttaagaacga gtatttgaat    240
aaaatagttg aatcaacctc agaaagccaa ctacagatac tgatgatgga gagtcgatca    300
aaagtagatg aagctgtgtc taagtttgaa aaggactcat cttcttcgtc aagttcagac    360
tcttccacta aaccggaagc ttcagataca gcgaagccaa acaagccgac agaaccagga    420
gaaaaggtag cagaagctaa gaagaaggtt gaagaagttg agaaaaaagc caaggatcaa    480
aaagaagaag atcgtcgtaa ctacccaacc aattacttac aaacgcttga acttgaaatt    540
gctgagtccg atgtggaagt taaaaaagcg gagcttgaac tagtaaaagt gaaagctaac    600
gaacctcgag acaagcaaaa aattaagcaa gcagaagcgg aagttgagag taaacaagct    660
gaggctacaa ggttaaaaaa aatcaagaca gatcgtgaag aagcagaaga agaagctaaa    720
cgaagagcag atgctaaaga gcaaggtaaa ccaaaggggc ggccaaaacg aggagttcct    780
ggagagctag caacacctga taaaaaagaa aatgatgcga agtcttcaga ttctagcgta    840
ggtgaagaaa ctcttccaag cccatccctg aaaccagaaa aaaaggtagc agaagctgag    900
aagaaggttg aagaagctaa gaaaaaagcc gaggatcaaa aagaagaaga tcgccgtaac    960
tacccaacca atacttacaa aacgcttgaa cttgaaattg ctgagtccga tgtggaagtt   1020
aaaaaagcgg agcttgaact agtaaaagag gaagctaagg aacctcgaaa cgaggaaaaa   1080
gttaagcaag caaaagcgga agttgagagt aaaaaagctg aggctacaag gttagaaaaa   1140
atcaagacag atcgtaaaaa agcagaagaa gaagctaaac gaaaagcagc agaagaagat   1200
aaagttaaag aaaaaccagc tgaacaacca caaccagcgc cggctccaaa aacagaaaaa   1260
ccagctccag ctccaaaacc agagaatcca gctgaacaac caaaagcaga aaaaccagct   1320
gatcaacaag ctgaagaa                                                 1338
```

<210> 24
<211> 1284
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA prepared from Streptococcus pneumoniae genome

<400> 24

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gaaggggtta gaagtgggaa taactccacg gttacatcta gtgggcaaga tatatcgaag     60
aagtatgctg atgaagtcga gtcgcatcta caaagtatat tgaaggatgt caataaaaat    120
ttgaagaaag ttcaacatac ccaaaatgcc gacttcaaca aaaagttgag caaaattaaa    180
acgaagtatt tgtatgaatt aaatgtttta gaagagaagt cggaagctga gttgacgtca    240
aaaacaaaag aaacaaaaga agagttaacc gcagcttttg agcagtttaa aaaagataca    300
ttatcaacag aaccagaaaa aaaggtagca gaagctaaga agaaggttga agaagctaag    360
aaaaaagccg aggatcaaaa agaaaaagat cgccgtaact acccaaccat tacttacaaa    420
acgcttgaac ttgaaattgc tgagtccgat gtggaagtta aaaaagcgga gcttgaacta    480
gtaaaagtga aagctaacga acctcgagac gaggaaaaaa ttaagcaagc agaagcgaaa    540
gttgagagta aacaagctga ggctacaagg ttaaaaaaaa tcaagacaga tcgtgaacaa    600
gctgaggcta caaggttaga aaacatcaag acagatcgtg aacaagcaga agaagaagct    660
aaagttaaag atgaaccaaa gaagcggaca aaacgaggag ttcttggaga gccagcaaca    720
cctgataaaa aagaaaatga tgcgaagtct tcagattcta gcgtaggtga agaaactctt    780
ccaagcccat ccctgaaacc agaaaaaaag gttgcagaag ctgagaagaa ggttgaagaa    840
gctaagaaaa aagccgagga tcaaaaagaa gaagatcgtc gtaactaccc aaccaatact    900
tacaaaacgc ttgaacttga aattgctgag tccgatgtgg aagttaaaaa agcggagctt    960
gaactagtaa aagaggaagc taaggaacct cgaaacgagg aaaaagttaa gcaagcaaaa   1020
gcggaagttg agagtaaaca agctgaggct acaaggttag aaaacatcaa gacagatcgt   1080
aaaaaagcag aagaagaagc taaacgaaaa gcagcagaag aagataaagt taaagaaaaa   1140
ccagctgaac aaccacaacc agcgccggct cctcaaccag aaaaaccagc tccaaaacca   1200
gaaaaaccag ctccagctcc aaaaccagag aatccagctg aacaaccaaa agcagaaaaa   1260
ccagctgatc aacaagctga agaa                                          1284
```

<210> 25
<211> 658

<212> DNA
<213> Artificial Sequence

<220>
<213> Description: cDNA derived from genome of Streptococcus pneumoniae

<400> 25
gaaggggtta gaagtgggaa taactccacg gttacatcta gtgggcaaga tatatcgaag 60
aagtatgctg atgaagtcga gtcgcatcta caaagtatat tgaaggatgt caataaaaat 120
ttgaaaaaag ttcaacatac ccaaaatgcc gacttcaaca aaaagttgag caaaattaaa 180
ccgaagtatt tgtatgaatt aaagtgttta gaagagaagt cggaagctga gttgacgtca 240
aaaccaaaga acaaaagaag agttaccgca gcttttgagc agtttaaaaa agatacatta 300
tcaacagaac cagaaaaaaa ggtagcagaa gctaagaaga aggttgaaga agctaagaaa 360
aaagccgagg atcaaaaaga aaaagatcgc cgtaactacc caaccattac ttacaaaacg 420
cttgaacttg aaattgctga gtccgatgtg gaagttaaaa aagcggagct tgaactagta 480
aaagaggaag ctaaggaacc tcgaaacgag gaaaaagtta agcaagcaaa agcggaagtt 540
gagagtaaac aagctgaggc tacaaggtta gaaaaaatca agacagatcg taaaaaagca 600
gaagaagaag ctaaacgaaa agcagcagaa gaagataaag ttaaagaaaa accagctg 658

<210> 26
<211> 1338
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA derived from genome of Streptococcus pneumoniae

<400> 26
acagagaacg agggagctac ccaagtaccc acttcttcta atagggcaaa tgaaagtcag 60
gcagaacaag gagaacaacc taaaaaactc gattcagaac gagataaggc aaggaaagag 120
gtcgaggaat atgtaaaaaa aatagtgggt gagagctatg caaaatcaac taaaaagcga 180
catacaatta ctgtagctct agttaacgag ttgaacaaca ttaagaacga gtatttgaat 240
aaaatagttg aatcaacctc agaaagccaa ctacagatac tgatgatgga gagtcgatca 300
aaagtagatg aagctgtgtc taagtttgaa aaggactcat cttcttcgtc aagttcagac 360
tcttccacta aaccggaagc ttcagataca gcgaagccaa acaagccgac agaaccagga 420
gaaaaggtag cagaagctaa gaagaaggtt gaagaagctg agaaaaaagc caaggatcaa 480
aaagaagaag atcgtcgtaa ctacccaacc attacttaca aaacgcttga acttgaaatt 540
gctgagtccg atgtggaagt taaaaaagcg gagcttgaac tagtaaaagt gaaagctaac 600
gaacctcgag acgagcaaaa aattaagcaa gcagaagcgg aagttgagag taaacaagct 660
gaggctacaa ggttaaaaaa aatcaagaca gatcgtgaag aagcagaaga agaagctaaa 720
cgaagagcag atgctaaaga gcaaggtaaa ccaaaggggc gggcaaaacg aggagttcct 780
ggagagctag caacacctga taaaaaagaa aatgatgcga agtcttcaga ttctagcgta 840
ggtgaagaaa ctcttccaag cccatccctg aaaccagaaa aaaaggtagc agaagctgag 900
aagaaggttg aagaagctaa gaaaaaagcc gaggatcaaa aagaagaaga tcgccgtaac 960
tacccaacca atacttacaa aacgcttgaa cttgaaattg ctgagtccga tgtggaagtt 1020
aaaaaagcgg agcttgaact agtaaaagag gaagctaagg aacctcgaaa cgaggaaaaa 1080
gttaagcaag caaaagcgga agttgagagt aaaaaagctg aggctacaag gttagaaaaa 1140
atcaagacag atcgtaaaaa agcagaagaa gaagctaaac gaaaagcagc agaagaagat 1200
aaagttaaag aaaaaccagc tgaacaacca caaccagcgc cggctccaaa agcagaaaaa 1260
ccagctccag ctccaaaacc agagaatcca gctgaacaac caaaagcaga aaaaccagct 1320
gatcaacaag ctgaagaa 1338

<210> 27
<211> 1242
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA derived from genome of Streptococcus pneumoniae

<400> 27
acagaaaacg aaggaagtac ccaagcagcc acttcttcta atatggcaaa gacagaacat 60
aggaaagctg ctaaacaagt cgtcgatgaa tatatagaaa aaatgttgag ggagattcaa 120
ctagatagaa gaaaacatac ccaaaatgtc gccttaaaca taaagttgag cgcaattaaa 180
acgaagtatt tgcgtgaatt aaatgtttta gaagagaagt cgaaagatga gttgccgtca 240
gaaataaaag caaagttaga cgcagctttt gagaagttta aaaaagatac attgaaacca 300
ggagaaaagg tagcagaagc taagaagaag gttgaagaag ctaagaaaaa agccgaggat 360
caaaaagaag aagatcgtcg taactaccca accaatactt acaaaacgct tgaacttgaa 420
attgctgagt tcgatgtgaa agttaaagaa gcggagcttg aactagtaaa agaggaagct 480
aaagaatctc gaaacgaggg cacaattaag caagcaaaag agaaagttga gagtaaaaaa 540
gctgaggcta caaggttaga aaacatcaag acagatcgta aaaaagcaga agaagaagct 600
aaacgaaaag cagatgctaa gttgaaggaa gctaatgtag cgacttcaga tcaaggtaaa 660
ccaaaggggc gggcaaaacg aggagttcct ggagagctag caacacctga taaaaaagaa 720
aatgatgcga agtcttcaga ttctagcgta ggtgaagaaa ctcttccaag ctcatccctg 780
aaatcaggaa aaaaggtagc agaagctgag aagaaggttg aagaagctga gaaaaaagcc 840
aaggatcaaa aagaagaaga tcgccgtaac tacccaacca atacttacaa aacgcttgac 900
cttgaaattg ctgagtccga tgtgaaagtt aaagaagcgg agcttgaact agtaaaagag 960
gaagctaagg aacctcgaga cgaggaaaaa attaagcaag caaaagcgaa agttgagagt 1020
aaaaaagctg aggctacaag gttagaaaac atcaagacag atcgtaaaaa agcagaagaa 1080
gaagctaaac gaaaagcagc agaagaagat aaagttaaag aaaaaccagc tgaacaacca 1140
caaccagcgc cggctactca accagaaaaa ccagctccaa aaccagagaa gccagctgaa 1200
caaccaaaag cagaaaaaac agatgatcaa caagctgaag aa 1242

<210> 28
<211> 1275
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA derived from genome of Streptococcus pneumoniae

<400> 28
acagagaagg aggtaactac ccaagtagcc acttcttcta atagggcaaa tgaaagtcag 60
gcaggacata ggaaagctgc tgaacaattc gatgaatata taaaaacaat gatccaatta 120
gatagaagaa aacataccca aaatttcgcc ttaaacataa agttgagcag aattaaaacg 180
gagtatttgc gtaaattaaa tgttttagaa gagaagtcga aagctgagtt gccgtcagaa 240
acaaaaaaag agatagacgc agcttttgag cagtttaaaa aagataccaa cagaaccaaa 300
aaaacggtag cagaagctga gaagaaggtt gaagaagcta agaaaaaagc caaggctcaa 360
aaagaagaag atcaccgtaa ctacccaacc aatacttaca aaacgcttga acttgaaatt 420
gctgagtccg atgtggaagt taaaaaagcg gagcttgaac tagtaaaaga ggaagctaag 480
gaatctcgag acgatgaaaa aattaagcaa gcagaagcga aagttgagag taaaaaagct 540
gaggctacaa ggttagaaaa catcaagaca gatcgtgaaa aagcagaaga agaagctaaa 600
cgaagagcag aagctaagtt gaaggaagct gttgaaaaga atgtagcgac ttcagagcaa 660
gataaaccaa aggggcggag aaaacgagga gttcctggag agcaagcaac acctgataaa 720
aaagaaaatg atgcgaagtc ttcagattct agcgtaggtg aagaagctct tccaagccca 780
tccctgaaac cagaaaaaaa ggttgcagaa gctgagaaga aggttgcaga agctgagaaa 840
aaagccaagg ctcaaaaaga agaagatcgc cgtaactacc caaccaatac ttacaaaacg 900
cttgaacttg aaattgctga gtccgatgtg aaagttaaag aagcggagct tgaactagta 960
aaagaggaag ctaaggaatc tcgaaacgag gaaaaagtta atcaagcaaa agcgaaagtt 1020
gagagtaaaa aagctgaggc tacaaggtta gaaaaaatca agacagatcg taaaaaagca 1080
gaagaagaag ctaaacgaaa agcagcagaa gaagataaag ttaaagaaaa accagctgaa 1140
caaccacaac cagcgccggc tcctcaacca gaaaaaccaa ctgaagagcc tgagaatcca 1200
gctcccgcac caaaaccaga gaagccagct gaacaaccaa aagcagaaaa aacagatgat 1260
caacaagctg aagaa 1275

<210> 29
<211) 1278
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: cDNA derived from genome of Streptococcus pneumoniae

<400> 29
acagagaagg aggtaactac ccaagtagcc acttcttcta ataaggcaaa taaaagtcag 60
acagaacata tgaaagctgc taaacaagtc gatgaatata taaaaaaaaa gctccaatta 120
gatagaagaa aacataccca aaatgtcggc ttactcacaa agttgggcgt aattaaaacg 180
gagtatttgc atggattaag tgtttcaaaa aagaagtcgg aagctgagtt gccgtcagaa 240
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aaaaaagctg aggctacaag gctagaaaac atcaagacag atcgtaaaaa agcagaagaa 1080
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ccacaaccag cgccggctcc tcaaccagaa aaaccaactg aagagcctga gaatccagct 1200
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gatcaacaag ctgaagaa 1278

<210> 30
<211> 1276
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<213> Artificial Sequence

<220>
<223> Description: cDNA derived from genome of Streptocossus pneumoniae

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gagtatttgc atggattaag tgtttcaaaa aagaagtcgg aagctgagtt gccgtcagaa 240
ataaaagcaa agttagacgc agcttttgag cagtttaaaa aagatacatt accaacagaa 300
ccaggaaaaa aggtagcaga agctgagaag aaggttgaag aagctaagaa aaaagccgag 360
gatcaaaaag aaaaagatct ccgtaactac ccaaccaata cttacaaaac gcttgaactt 420
gacattgctg agtccgatgt ggaagttaaa aaagcggagc ttgaactagt aaaagaggaa 480
gctaaggaat ctcgagacga gaaaaaaatt aatcaagcaa aagcgaaagt tgagaataaa 540
aaagctgagg ctacaaggtt aaaaaacatc aagacagatc gtgaaaaagc agaagaagct 600
aaacgaagag cagatgctaa gttgcaggaa gctaatgtag cgacttcaga gcaagataaa 660
tcaaagaggc gggcaaaacg agaagttttt ggagagctag caacacctga taaaaaagaa 720
aatgatgcga agtcttcaga ttctagcgta ggtgaagaaa ctcttacaag cccatccctg 780
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gaagctaagg aatctcgaaa cgaggaaaaa attaagcaag taaaagcgaa agttgagagt 1020
aaaaaagctg aggctacaag gctagaaaac atcaagacag atcgtaaaaa agcagaagaa 1080
gaagaagcta aacgaagagc agcagaagaa gataaagtta aagaaaaacc agctgaacaa 1140
ccacaaccag cgccggctcc tcaaccagaa aaaccaactg aagagcctga gaatccagct 1200
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gatcaacaag ctgaag 1278

<210> 31
<211> 1272
<212> DNA

<213> Artificial Sequence

<220>
<223> Descritpion: cDNA derived from genome of Streptococcus pneumoniae

<400> 31
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gatagaagaa aacataccca aaatgtcggc ttactcacaa agttgggcgt aattaaaacg     180
gagtatttgc atggattaag tgtttcaaaa aagaagtcgg aagctgagtt gccgtcagaa     240
ataaaagcaa agttagacgc agcttttgag cagtttaaaa aagatacatt accaacagaa     300
ccaggtaaaa aggtagcaga agctgagaag aaggttgaag aagctaagaa aaaagccgag     360
gatcaaaaag aaaaagatct ccgtaactac ccaaccaata cttacaaaac gcttgaactt     420
gacattgctg agtccgatgt ggaagttaaa aaagcggagc ttgaactagt aaaagaggaa     480
gctaaggaat ctcgagacga gaaaaaaatt aatcaagcaa aagcgaaagt tgagaataaa     540
aaagctgagg ctacaaggtt aaaaaacatc aagacagatc gtgaaaaagc agaagaagct     600
aaacgaagag cagatgctaa gttgcaggaa gctaatgtag cgacttcaga gcaagataaa     660
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gatcaacaag ct                                                        1272
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<210> 32
<211> 1258
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: Coding strand of cDNA derived from genome of Streptococcus pneumoniae

<400> 32
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aaacataccc aaaatttagc cttcaacata cagttgagca gaattaaaac ggagtatttg     180
aatggattaa aagagaagtc ggaagctgag ttgccgtcaa aaataaaagc agagttagac     240
gcagctttta agcagtttaa aaaagataca ttaccaacag aaccagaaaa aaaagtagca     300
gaagctgaga agaaggttga agaagctgag aagaaggtag cagaagctaa gaaaaaagcc     360
aaggctcaaa aagaagaaga tcaccgtaac tacccaacca ttacttacaa aacgcttgac     420
cttgaaattg ctgagttcga tgtgaaagtt aaagaagcgg agcttgaact agtaaaaaag     480
gaagctgacg aatctcgaaa cgagggcaca attaaccaag caaaagcgaa agttgagagt     540
gaaaaagctg aggctacaag gttaaaaaaa atcaagacag atcgtgaaaa agcagaagaa     600
gaagaagcta aacgaagagc agatgctaaa gagcaagatg aatcaaagag gcgaaagagt     660
cggggaaaac gaggagctct tggagagcaa gcaacacctg ataaaaaaga aaatgatgcg     720
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gctgagtccg atgtgaaagt taaagaagcg gagcttgaac tagtaaaaga ggaagctaag     960
gaatctcgaa acgaggaaaa aattaagcaa gcaaaagcga aagttgagag taaaaaagct    1020
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cgaaaagcag cagaagaaga taaagttaaa gaaaaaccag ctgaacaacc acaaccagcg    1140
ccggctcctc aaccagaaaa accagctgaa gagcctgaga atccagttcc agctccaaaa    1200
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<210> 33
<211> 1242
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: Coding strand of cDNA derived from genome of Streptococcus pneumoniae

<400> 33
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ctagatagaa gaaaacatac ccaaaatgtc gccttaaaca taaagttgag cgcaattaaa     180
acgaagtatt tgcgtgaatt taatgtttta gaagagaagt cgaaggatga gttgccgtca     240
gaaataaaag caaagttaga cgcagctttt gagaagttta aaaaagatac attgaaacca     300
ggagaaaagg tagcagaagc taagaagaag gttgaagaag ctaagaaaaa agccgaggat     360
caaaaagaag aagatcgtcg taactaccca accaatactt acaaaacgct tgaacttgaa     420
attgctgagt tcgatgtgaa agttaaagaa gcggagcttg aactagtaaa agaggaagct     480
aaagaatctc gaaacgaggg cacaattaag caagcaaaag agaaagttga gagtaaaaaa     540
gctgaggcta caaggttaga aaacatcaag acagatcgta aaaaagcaga agaagaagct     600
aaacgaaaag cagatgctaa gttgaaggaa gctaatgtag cgacttcaga tcaaggtaaa     660
ccaaaggggc gggcaaaacg aggagttcct ggagagctag caacacctga taaaaaagaa     720
aatgatgcga agtcttcaga ttctagcgta ggtgaagaaa ctcttccaag ctcatccctg     780
aaatcaggaa aaaaggtagc agaagctgag aagaaggttg aagaagctga gaaaaaagcc     840
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<210> 34
<211> 1236
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: Coding strand from cDNA derived from genome of Streptococcus pneumoniae

<400> 34
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ttagatagaa gtaaacatat caaaactgta aatctaatta acaaattgca agacattaag     180
agaacgtatt tgtatgaatt aaatgtttta gaagataagt cgaaagctga gttgccgtca     240
aaaataaaag cagagttaga cgcagctttt gagcagttta aaaaagatac attaccaaca     300
gaaccaggaa aaaaggtagc agaagctaag aagaaggttg aagaagctga gaaaaaagcc     360
aaggctcaaa aagaagaaga ttaccgtaac tacccaacca ttacttacaa aacgcttgaa     420
cttgaaattg ctgagtccga tgtgaaagtt aaagaagcgg agcttgaact agtaaaaaag     480
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gaagctaaac gaagagcaga tgctaaagag caagatgaat caaagaggcg aaagagtcgg     660
gtaaaacgag gagattttgg agagccagca acacctgata aaaaagaaaa tgatgcgaag     720
tcttcagatt ctagcgtagg tgaagaaact cttccaagcc catccctgaa accaggaaaa     780
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gctcctcaac cagaaaaacc agctccagct ccaaaaccag agaatccagc tgaacaacca 1200
aaagcagaaa aaccagctga tcaacaagct gaagaa 1236

<210> 35
<211> 1218
<212> DNA
<213> Artificial Sequence

<220>
<223> Description: Coding strand from cDNA derived from genome of Streptococcus
pneumoniae

<400> 35
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gagtatttgc gtggattaag tgtttcaaaa gagaagtcga cagctgagtt gccgtcagaa 240
ataaaagaaa agttaaccgc agcttttaag cagtttaaaa aagatacatt gaaaccagaa 300
aaaaaggtag cagaagctga gaagaaggta gcagaagcta agaaaaaagc cgaggatcaa 360
aaagaagaag atcgtcgtaa ctacccaacc attacttaca aaacgcttga acttgaaatt 420
gctgagtccg atgtggaagt taaaaaagcg gagcttgaac tagtaaaagt gaaagctaac 480
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gaggctacaa ggttaaaaaa aatcaagaca gatcgtgaaa aagcagaaga agaagctaaa 600
cgaagagtag atgctaaaga gcaagatgaa tcatcaaaga ggcgaaagag tcgggtaaaa 660
cgaggagatc ttggagagca agcaacacct gataaaaaag aaaatgatgc gaagtcttca 720
gattctagcg taggtgaaga aactcttcca agcccatccc tgaaaccagg aaaaaaggta 780
gcagaagctg agaagaaggt tgaagaagct gataaaaaag ccaaggctca aaaagaagaa 840
gatcgccgta actacccaac caatacttac aaaacgcttg aacttgaaat tgctgagtcc 900
gatgtggaag ttaaaaaagc ggagcttgaa ctagtaaaag aggaagctaa ggaacctcga 960
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gcagaagaag ataaagttaa agaaaaacca gctgaacaac caaaaccagc gccggctcct 1140
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ccagctgatc aacaagct 1218

<210> 36
<211> 102
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from consensus cDNA sequence

<400> 36
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1 5 10 15

Asn Gln Thr Glu His Arg Lys Ala Ala Lys Gln Val Val Asp Glu Tyr
20 25 30

Ile Lys Lys Met Leu Glu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Glu Tyr Leu Arg
50 55 60

Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Ala Glu Leu Pro Ser Glu

65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
85 90 95

Leu Lys Thr Glu Pro Gly
100

<210> 37
<211> 55
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA consensus sequence from the genome of Streptococcus pneumoniae

<400> 37
Asp Ala Lys Leu Glu Ala Thr Ser Glu Gln Asp Lys Pro Lys Gly Arg
1 5 10 15

Ala Lys Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
20 25 30

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro
35 40 45

Ser Pro Ser Leu Lys Pro Glu
50 55

<210> 38
<211> 103
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from consensus sequence of cDNA
derived from the genome of Streptococcus pneumoniae

<400> 38
Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Lys Lys Lys
1 5 10 15

Ala Lys Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Ile Thr
20 25 30

Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys
35 40 45

Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser Arg Asp
50 55 60

Glu Gly Lys Ile Lys Gln Ala Lys Ala Lys Val Glu Ser Lys Lys Ala
65 70 75 80

Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg Glu Lys Ala Glu
85 90 95

Glu Glu Ala Lys Arg Arg Ala
100

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(57) Abstract

The present invention provides bacterial immunogenic agents for adminstration to humans and non-human animals to stimulate an immune response. It particularly relates to the vaccination of mammalian species with pneumococcal derived polypeptides that include an alpha helix but exclude a choline binding region as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. In another aspect the invention provides antibodies against such proteins and protein complexes that may be used as diagnostics and/or as protective/treatment agents for pathogenic bacterial species.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/07680

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/09 A61K39/40 C07K16/12 C07K14/315

According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 41151 A (UNIV ROCKEFELLER) 6 November 1997 (1997-11-06) page 8, line 3 -page 15, line 17 page 106, paragraphs SEQ.ID.20,,21 -page 108 ---	1-21
X	WO 97 09994 A (UAB RESEARCH FOUNDATION) 20 March 1997 (1997-03-20) page 6, line 12 -page 8, line 31 figures 13,21 ---	1-10,20, 21
X,P	WO 98 21337 A (SMITH BEVERLY ;CHENG QI (US); FINKEL DAVID J (US); UNIV MINNESOTA) 22 May 1998 (1998-05-22) page 4, line 8 -page 9, line 27 page 38, paragraphs SEQ.,ID.5,6 -page 42 --- -/--	1-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1999	26/10/1999
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Rempp, G

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International Application No
PCT/US 99/07680

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 98 18930 A (HUMAN GENOME SCIENCES INC ;CHOI GIL H (US); HROMOCKYJ ALEX (US); J) 7 May 1998 (1998-05-07) page 3, line 15 -page 6, line 21 page 55, paragraphs SEQ.,ID.,37,38 -page 56 -----	1-21

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/07680

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 19
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/07680

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 19 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 19

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/07680

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9741151	A	06-11-1997	AU	2818297	A	19-11-1997
			EP	0912608	A	06-05-1999
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			EP	0942984	A	22-09-1999
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			EP	0941335	A	15-09-1999
			WO	9818931	A	07-05-1998

09/056,019